



THE JOURNAL  
OF  
CLINICAL INVESTIGATION

VOLUME VII  
1929

PUBLISHED BY  
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION



## CONTENTS

### NUMBER 1, APRIL, 1929

|  |   |     |
|--|---|-----|
| The Output of the Heart in Patients with Abnormal Blood Pressures  | C<br>SIDNEY BURWELL AND W. CARTER SMITH   | 1   |
| The Effect of the Digitalis Bodies on the Velocity of Blood Flow Through the Lungs and on Other Aspects of the Circulation | A Study of Normal Subjects and Patients with Cardiovascular Disease<br>SOMA WEISS AND<br>HERMANN L. BLUMGART  | 11  |
| Observations on Paroxysmal Hemoglobinuria  | G. M. MACKENZIE   | 27  |
| A Quantitative Method for the Estimation of Pepsin   | W. SCOTT POLLAND<br>AND ARTHUR L. BLOOMFIELD  | 45  |
| Quantitative Measurements of Pepsin in Gastric Juice Before and After Histamine Stimulation.                               | W. SCOTT POLLAND AND ARTHUR L. BLOOMFIELD   | 57  |
| Studies of Calcium and Phosphorus Metabolism   | II The Calcium Excretion of Normal Individuals on a Low Calcium Diet Including Data on a Case of Pregnancy<br>WALTER BAUER, FULLER ALBRIGHT AND JOSEPH C. AUB | 75  |
| Studies of Calcium and Phosphorus Metabolism   | III The Effects of the Thyroid Hormone and Thyroid Disease<br>JOSEPH C. AUB, WALTER BAUER, CLARE HEATH AND MARION ROPES                                       | 97  |
| Studies of Calcium and Phosphorus Metabolism   | IV The Effects of the Parathyroid Hormone<br>FULLER ALBRIGHT, WALTER BAUER, MARION ROPES AND JOSEPH C. AUB  | 139 |

### NUMBER 2 JUNE 1929

|   |     |
|---|-----|
| Studies on the Physiology of the Parathyroid Glands I Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism<br>FULLER ALBRIGHT AND READ ELLSWORTH | 183 |
| The Determination of the Circulating Blood Volume in Infants by the Carbon Monoxide Method RUSTIN MCINTOSH  | 203 |
| An Apparatus for the Prolonged Administration of Artificial Respiration I A Design for Adults and Children. PHILIP DRINKER AND LOUIS A SHAW                           | 229 |
| The Involution of Cutaneous Xanthomata Caused by Diets Low in Calories A C CURTIS, U J WILE AND H C ECKSTEIN  | 249 |
| The Surface Tension of Blood Serum, and the Determination of the Surface Tension of Biological Liquids HENRY N HARKINS AND WILLIAM D HARKINS                          | 263 |

|  |  |     |
|--|--|-----|
| Blood Volume Preceding and Following Splenectomy in Hemolytic Icterus and Splenic Anemia                               | HERBERT Z GIFFIN GEORGE E BROWN AND GRACE M ROTH | 283 |
| Proceedings of the First Meeting of the Central Society for Clinical Research held in Chicago, Ill., November 23, 1928 |  | 303 |

## NUMBER 3, AUGUST 1929

|  |   |     |
|--|---|-----|
| Studies of Serum Electrolytes IV The Chloride and Nitrogen Balances, and Weight Changes in Pneumonia   | F WILLIAM SUNDERMAN   | 313 |
| Studies of Serum Electrolytes V Urinary Electrolyte Excretion in Pneumonia   | J HAROLD AUSTIN AND F WILLIAM SUNDERMAN   | 333 |
| The Effect of Exercise on the Size of Normal Hearts and of Enlarged Hearts of Dogs   | HAROLD J STEWART  | 339 |
| Metabolism of Chloride and Total Fixed Base in Pneumonia and the Relation to Salt and Water Retention  | T S WILDER AND T G H DRAKE  | 353 |
| An Investigation of Various Factors Which Affect the Sedimentation Rate of the Red Blood Cells   | M DOROTHY ROURKE AND E D PLASS  | 365 |
| Observations on the Effect of Various Factors on the Duration of the Electrical Systole of the Heart as Indicated by the Length of the Q-T Interval of the Electrocardiogram | PAUL D WHITE AND SEELEY G MUDD  | 387 |
| The Calorigenic Action of Thyroxin at Different Levels of Basal Metabolism in Myxedema   | WILLARD OWEN THOMPSON, PHEBE K THOMPSON, ALLEN G BRAILEY, AND ARCHIBALD C COHEN | 437 |
| The Action of Sodium Chloride, Ammonium Chloride, and Sodium Bicarbonate on the Total Acid-base Balance of a Case of Chronic Nephritis with Edema                            | FULLER ALBRIGHT AND WALTER BAUER  | 465 |
| Proceedings of the Twenty-first Annual Meeting of the American Society for Clinical Investigation Held in Atlantic City, N J, May 6, 1929                                    |   | 487 |

## NUMBER 4, OCTOBER, 1929

|  |  |     |
|--|--|-----|
| The Influence of the Sympathetic Nervous System on the Capillaries during Passive Congestion | J HAMILTON CRAWFORD  | 527 |
| The Excretion of Zinc in Health and Disease  | LAWRENCE T FAIRHALL AND LYMAN H HOYT                       | 537 |
| Skin Reactions to Filtrates of Haemolytic Streptococci in Acute and Subacute Nephritis       | OSCAR C HANSEN-PRUSS, WARFIELD T LONGCOPE, AND D P O'Brien | 543 |
| The Color of the Skin as Analyzed by Spectrophotometric Methods I Apparatus and Procedures   | CHARLES SHEARD AND LOUIS A BRUNSTING                       | 559 |
| The Color of the Skin as Analyzed by Spectrophotometric Methods II The Role of Pigmentation  | LOUIS A BRUNSTING AND CHARLES SHEARD                       | 575 |

|   |   |  |
|---|---|--|
| The Color of the Skin as Analyzed by Spectrophotometric Methods | III   |  |
| The Rôle of Superficial Blood                                   | LOUIS A. BRUNSTING AND CHARLES SHEARD   | 593  |
| Studies on Red Blood Cell Diameter                              | III The Relative Diameter of Immature (Reticulocytes) and Adult Red Blood Cells in Health and Anemia, Especially in Pernicious Anemia | ELBERT LAPSLEY PERSONS 615                     |
| Studies on Red Blood Cell Diameter                              | IV The Decrease in the Mean Diameter of the Reticulocytes and Adult Red Blood Cells in Pernicious Anemia Following Liver Therapy      | GREENE FITZHUGH AND ELBERT LAPSLEY PERSONS 631 |
| Index to Volume VII   |   | 637  |



## THE OUTPUT OF THE HEART IN PATIENTS WITH ABNORMAL BLOOD PRESSURES

BY C. SIDNEY BURWELL AND W. CARTER SMITH

(From the Medical Clinic, Vanderbilt University Hospital, Nashville)

(Received for publication September 18, 1928)

Heart failure is preceded by hypertension in a considerable percentage of cases. O'Hare, Calhoun and Altnow (1) have recently shown that many patients with heart failure of the type called "chronic myocarditis" or "myocardial insufficiency of unknown origin" have had high blood pressure in the past. In any attempt to analyze the effects of high systemic arterial pressure and to evaluate it as a factor in the causation of heart failure, it is desirable to have knowledge of the other dynamic factors known to be concerned in the work of the heart. The work of the heart is determined chiefly by its output of blood per minute, by the pressure in the pulmonary artery and in the aorta against which it pumps, and by the velocity imparted to the blood by the heart. In the absence of greatly increased output the velocity factor is small relative to the other two, although, as Evans (2) has shown, when large outputs are concerned it may be of major importance.

Blumgart and Weiss (3) have studied the velocity of blood flow in patients with arterial hypertension. They found that patients with hypertension who exhibited no evidence of circulatory disability could be divided into two groups: in one, the velocity of blood flow was within the limits of normal, in the other the velocity was retarded.

Cardiac output in patients with arterial hypertension has been studied by Plesch (4), by Liljestrand and Stenström (5), and by Haya-saka (6). Plesch's results in four cases showed on the average no definite variation in either direction. Liljestrand and Stenström made repeated observations of the cardiac outputs of five women suffering from increased blood pressure and chronic nephritis. They used the nitrous oxide method of Krogh and Lindhard (7) and found

that the average cardiac output of these patients was increased 26 per cent above the average of a group of normal subjects. The basal metabolic rate was correspondingly on the average 17 per cent higher than in the control group. The highest outputs were in cases with moderately elevated pressures rather than in those with extremely high ones.

The work of Hayasaka, which was done by the triple-extrapolation method of Redfield, Bock, and Meakins (8), gave somewhat confusing results. He used the output per minute per square meter of body surface as the standard of comparison, a value which Burwell and Robinson (9) have shown to be an uncertain one, and found the cardiac output increased in "benign hypertension" and in "malignant hypertension with nephritis," but unchanged in cases with "secondary contracted kidney." No data concerning variations in metabolic rate are given, but the experiments were performed under standard conditions. The author is of the opinion that hypertension in these cases may be dependent upon the elevated cardiac output, but he also points out that increased blood pressure is not always accompanied by increase in cardiac output.

#### METHODS

The observations here reported were made by the method of Field, Bock, Gildea and Lathrop (10) with careful attention to the constancy of "standard basal" conditions as described in previous publications (11). In each case, observations were made only after several days of rest in hospital had led to the establishment of a relatively fixed level of blood pressure. Whenever possible, more than one observation was made on each subject. To emphasize the ease with which this method may be applied to patients untrained in respiratory methods and as evidence for the reliability of the results obtained there is presented in table 1 a record of all the differences in arterial and venous carbon dioxide tension observed in these experiments. It will be noted that in each experiment the arterio-venous differences agree satisfactorily, usually within 1 mm and that the maximum variation is 1.7 mm.

Five patients with arterial hypertension were studied, three men and two women. The systolic pressures ranged between 175 mm and

225 mm mercury and the diastolic between 110 mm and 140 mm. Although we attempted to select relatively uncomplicated cases all of the patients, as may be seen from the appended clinical summaries, showed evidence of more or less arteriosclerosis and several of them had undoubted chronic nephritis. One patient (case 5) had had a partial thyroidectomy four months before our study was made, and at the time of observation had a slightly elevated basal metabolic rate. No patient had evidence of congestive heart failure at the time of observation and only one (case 3) had a history suggesting its presence previously. All patients in the group showed definite evidence of cardiac enlargement in the teleoroentgenogram or by per-

TABLE 1  
*Arterio-venous differences in carbon dioxide tension (millimeters of mercury)*

| Subject number    | 1    | 2   | 2   | 3   | 3   | 4   | 4   | 5   | 5   | 6   | 7   | 7   | 8   | 9    |
|-------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| Experiment number | 1    | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14   |
| 1                 | 11.1 | 7.8 | 6.3 | 8.1 | 9.8 | 9.9 | 8.2 | 8.6 | 7.4 | 7.6 | 7.8 | 6.8 | 8.0 | 11.9 |
| 2                 | 10.3 | 7.4 | 6.9 | 7.9 | 9.8 | 8.4 | 9.9 | 8.4 | 7.3 | 7.3 | 7.7 | 8.3 | 8.0 | 11.7 |
| 3                 | 11.5 | 6.8 | 7.1 | 8.7 | 8.9 | 9.2 | 9.3 | 8.7 | 7.2 | 7.5 | 7.2 | 7.1 | 7.9 | 10.9 |
| 4                 |      |     |     | 7.5 | 9.6 | 8.6 | 8.6 | 8.3 | 7.4 | 7.4 |     |     |     | 11.5 |
| 5                 |      |     |     |     |     | 8.8 | 9.0 | 8.3 |     |     |     |     |     |      |
| Average           | 10.9 | 7.3 | 6.8 | 8.1 | 9.5 | 8.9 | 9.0 | 8.5 | 7.3 | 7.5 | 7.6 | 7.4 | 8.0 | 11.4 |

cussion. In four subjects a second estimation of cardiac output was made after an interval of one or more days.

Similar observations were carried out upon four men exhibiting systolic blood pressures of from 65 mm to 95 mm of mercury and diastolic pressures ranging from 55 mm to 70 mm. Of these, one was a case of rapidly progressive Addison's disease, in which the diagnosis was subsequently confirmed by autopsy. To within 48 hours of our observation he had been receiving frequent injections of adrenalin and also whole dried adrenal gland by mouth. Another subject was a young man suffering from tuberculosis of the lungs and cecum and probably Addison's disease. Two measurements were made of this patient's cardiac output. Of the two other subjects, Cases 8 and 9, one was a member of the hospital staff and the other a medical student,

both apparently quite normal, but having low systolic blood pressure under the imposed conditions of fasting and rest

For convenience in comparison certain average values are calculated. In order to avoid distortion because some patients have two and some only one set of figures, the averages are obtained by calculating first the average values for each subject and then computing the average of these figures

### RESULTS

The results of the studies are given in table 2. The cardiac output of all the subjects in both groups falls within the limits seen in normal healthy people when the blood pressures are within the usual zone. The averages of the cardiac outputs in the two groups are almost identical and the average cardiac output per minute per kilogram of body weight and the average output of the heart per beat are of the same general order. The absence of variation is striking when it is observed that the average pulse pressure in the hypertensive group is 85 mm. as against 26 mm. in the hypotensive group.

The basal metabolic rate is on the whole somewhat higher in the group with high pressures than in the group with low. This is partly explicable on the score of the elevated rate of subjects 4 and 5, on the one hand, and the characteristically low rate of the patient with Addison's disease, on the other. The cardiac output per 100 cc oxygen absorbed is, however, of the same order in the two groups. The pulse rates in both groups with the exception of those of the two normal men are slightly above the low level usually attained under satisfactory "basal" conditions, and the average rate is higher in the hypertensive cases, just as is the metabolic rate. The arterio-venous differences do not differ significantly in the two groups.

### DISCUSSION

These observations demonstrate that under conditions of bodily rest patients with arterial hypertension have no significant increase in the cardiac output. The hypothesis that hypertension in general is secondary to an increased output is thus not tenable. An admissible objection to this conclusion is the possibility that the cardiac output was increased earlier in the history of these patients but that at the

TABLE 2  
The cardiac output and related data in patients with high and low blood pressures

| Date                             | Case number | Experiment number | Blood pressure |             | Pulse pressure | Vital capacity |                 | Arterio-venous difference | Carbon dioxide per minute | Cardiac output per minute | Cardiac output per kilo per minute | Cardiac output per 100 cc. oxygen | Cardiac output per beat | Pulse rate | Respiratory quotient | Basal metabolic rate | Weight in kilos |
|----------------------------------|-------------|-------------------|----------------|-------------|----------------|----------------|-----------------|---------------------------|---------------------------|---------------------------|------------------------------------|-----------------------------------|-------------------------|------------|----------------------|----------------------|-----------------|
|                                  |             |                   | Systolic       | Diastolic   |                | Actual         | Per cent normal |                           |                           |                           |                                    |                                   |                         |            |                      |                      |                 |
|                                  |             |                   | mm. mercury    | mm. mercury | mm. mercury    | cc.            |                 | mm. mercury               | cc.                       | cc.                       | cc.                                | cc.                               | cc.                     | per min.   |                      | per cent             |                 |
| February 13 1928                 | 1           | 1                 | 215            | 125         | 90             | 3,700          | 71              | 4 58                      | 195                       | 4,250                     | 58                                 | 1,740                             | 51                      | 84         | 0 80                 | +3                   | 73              |
| February 18 1928                 | 2           | 2                 | 205            | 110         | 95             | 2,100          | 75              | 3 12                      | 118                       | 3,780                     | 88                                 | 2,380                             | 49                      | 77         | 0 74                 | -7                   | 43              |
| February 20 1928                 | 3           | 3                 | 200            | 110         | 90             | 2,100          | 75              | 2 90                      | 126                       | 4 350                     | 101                                | 2,560                             | 59                      | 74         | 0 76                 | -4                   | 43              |
| April 12, 1928                   | 3           | 4                 | 187            | 130         | 57             | 3 000          | 72              | 3 34                      | 176                       | 5,270                     | 83                                 | 2 150                             | 68                      | 78         | 0 72                 | +2                   | 63              |
| April 16 1928                    | 4           | 5                 | 172            | 120         | 50             | 2 600          | 61              | 3 96                      | 164                       | 4,140                     | 66                                 | 1,960                             | 54                      | 76         | 0 78                 | -11                  | 63              |
| April 29, 1928                   | 4           | 6                 | 225            | 140         | 85             | 3,400          | 91              | 3 70                      | 199                       | 5,380                     | 96                                 | 2,270                             | 63                      | 85         | 0 84                 | +20                  | 56              |
| April 30 1928                    | 5           | 7                 | 225            | 140         | 85             | 4 000          | 107             | 3 74                      | 196                       | 5,240                     | 94                                 | 2,160                             | 62                      | 85         | 0 81                 | +22                  | 56              |
| June 20, 1928                    | 5           | 8                 | 175            | 120         | 55             | 2,500          | 91              | 3 63                      | 173                       | 4 770                     | 95                                 | 2,120                             | 44                      | 108        | 0 77                 | +25                  | 50              |
| June 22, 1928                    |             | 9                 | 190            | 120         | 70             | 2 500          | 91              | 3 13                      | 174                       | 5,560                     | 111                                | 2 530                             | 58                      | 96         | 0 79                 | +22                  | 50              |
| Average in hypertensive patients |             |                   |                |             | 85             |                |                 | 3 65                      |                           | 4 700                     | 85                                 | 2,160                             | 56                      | 84         |                      | +7                   | 57              |
| December 7, 1927                 | 6           | 10                | 65             | 45          | 20             | 4,300          | 93              | 3 78                      | 166                       | 4 390                     | 70                                 | 1 960                             | 52                      | 84         | 0 83                 | -17                  | 63              |
| February 24, 1928                | 7           | 11                | 85             | 55          | 30             | 3 300          | 88              | 3 27                      | 164                       | 5 040                     | 114                                | 2,230                             | 55                      | 92         | 0 72                 | +8                   | 44              |
| March 3, 1928                    | 8           | 12                | 80             | 55          | 35             | 3,200          | 85              | 3 17                      | 158                       | 5 000                     | 114                                | 2 270                             | 57                      | 88         | 0 72                 | +4                   | 44              |
| March 18, 1928                   | 8           | 13                | 82             | 65          | 17             | 4 100          | 86              | 3 15                      | 178                       | 5,650                     | 83                                 | 2,600                             | 84                      | 66         | 0 82                 | -15                  | 68              |
| April 14, 1928                   | 9           | 14                | 95             | 60          | 35             | 5 000          | 100             | 5 01                      | 198                       | 3,950                     | 47                                 | 1 650                             | 62                      | 64         | 0 83                 | -13                  | 84              |
| Average in hypotensive patients  |             |                   |                |             | 26             |                |                 | 3 75                      |                           | 4 750                     | 75                                 | 2,075                             | 64                      | 76         |                      | -10                  | 65              |

time of observation they had reached a stage of the disease when the heart could no longer maintain so great an increase. Against the possibility of this it may be said that these were patients without signs or symptoms of heart failure and capable without distress of degrees of exertion certainly demanding greater cardiac output.

These observations show also that the increase in the work of the heart leading to its hypertrophy in patients with actual hypertension is due not to changes in velocity or cardiac output but to increase in resistance, unless exercise in hypertensive patients is accompanied by a disproportionate increase in output. Holman and Beck (12) from a comparison of the heart weights in two similar dogs, one of which had pulmonic stenosis and the other a large septal defect, infer that increased flow of blood through the heart is a more potent stimulus to hypertrophy and dilatation than increased peripheral resistance. The stimulus in our cases, however, was apparently increased resistance since no elevation of the cardiac output was observed.

A comparison of the hypertensive and hypotensive groups shows a striking lack of relationship between blood pressure and cardiac output. Here are patients with very high and very low systolic pressures, and with great variations in pulse pressure, but with nearly identical cardiac outputs. Blumgart and Weiss (3) pointed out the fact that hypertension does not necessarily imply any change in the velocity of blood flow. If increase in tension were necessarily followed by increase in cardiac output, or by increased velocity, the work of the heart would be still further increased to its further hurt. These observations must not be thought to show that hypertension and greatly increased cardiac output are never associated. They often are, as with the development of severe anemia in chronic nephritis with hypertension. And hypotension may, for example, be associated with distinctly diminished cardiac output in animals in shock as Blalock (13) has shown. The association of hypertension and increased output, or hypotension and decreased output, is accordingly not a necessary one.

These studies bear on the question of proportionality between pulse pressure and output per beat, a relation suggested by Erlanger and Hooker (14), who were fully aware of its limitations. Rosen and White (15) have recently brought evidence to show that the pulse

pressure is directly proportional to the output per beat in consecutive observations in the same individual under conditions that produce essentially the same diastolic pressure and heart rate. Our experiments show a complete lack of proportionality in different individuals, a lack of correspondence due, as Wiggers (16) points out, to variations in vascular elasticity and tonicity, the viscosity of the blood, and other factors

#### SUMMARY

In two groups of cases, one with systolic blood pressures of over 175 mm of mercury and one with systolic blood pressures of less than 95 mm of mercury, the total cardiac output per minute, the cardiac output per minute per kilogram of body weight and the cardiac output per 100 cc of oxygen absorbed did not show significant differences. Pulse pressure and output per beat are not directly proportional in different individuals

#### PROTOCOLS

1 F H, a white male age 59 years, complained of headache, dizziness, impaired vision, weakness and impairment of memory for 5 years. He was known to have had an elevated blood pressure for 5 years, and nocturia seven to eight times for 1 year. On physical examination he was a plethoric individual, with advanced arterio-sclerosis and vascular retinitis. The cardiac dulness was 11.5 cm to the left and 4 cm to the right. The heart sounds were of good quality, no murmurs were heard. There was no pitting edema or other evidence of cardiac failure. There were albuminuria and cylindruria but no nitrogen retention.

The diagnosis was hypertension, arterio-sclerosis, chronic nephritis.

2 E T, a colored woman of 55 years, had palpitation of the heart, weakness, and dyspnea on exertion for five years. There had been some precordial pain during one year. Examination showed a small, fairly well nourished colored woman. The eyegrounds showed vascular retinitis with retinal hemorrhages. The cardiac dulness was 9.0 cm to the left and 4.0 cm. to the right and the supra cardiac dulness was 5.8 cm wide. There were no evidences of cardiac failure, no anemia, and no evidence of nephritis.

The diagnosis was arterio-sclerosis, hypertension.

3 E C, a colored male aged 36 years complained of frequent attacks of palpitation of the heart for 14 months and some nocturnal dyspnea for 10 years.

He had a primary syphilitic lesion 17 years ago. On physical examination he was well nourished and developed. Examination of the eyegrounds showed an early arteriosclerosis. The teleoroentgenogram of the heart showed it to be 10.3 cm to the left, 3.7 cm to the right, and the aortic shadow was 6.4 cm in diameter. The heart sounds were of good quality, there was a soft systolic murmur at the apex but no evidence of cardiac failure. There was very little peripheral sclerosis and no evidence of renal disease. The blood Wasserman was positive.

The diagnosis was hypertension, early vascular disease, syphilis.

4 A J, a white male, aged 41 years, was known to have had hypertension and chronic nephritis for two and a half years. Headache was the most persistent and severe symptom. Examination showed a small fairly well nourished man with no evidence of cardiac failure but with marked peripheral arterial sclerosis and eyeground changes. A seven foot plate of the heart showed it to be 8.5 cm to the left, 3.6 cm to the right, and the aortic shadow was 6.5 cm. The heart sounds were of poor quality, but there were no murmurs. The blood contained 41 mgm of non-protein nitrogen per 100 cc. there was constant albuminuria, and the specific gravity of the urine was low and fixed. There was no anemia.

The diagnosis was chronic nephritis, hypertension, and arteriosclerosis.

5 F B, a white female of 26 years, admitted with a history of thyrotoxicosis four months previous to this entry. Thyroidectomy was performed at this time, this was followed by improvement but the blood pressure, which was known to have been elevated for seven months, remained high. She developed palpitation of the heart and slight dyspnea on exertion. On physical examination she was rather poorly nourished but there were no definite signs of thyrotoxicosis. The cardiac dulness was 9.5 cm to the left. There was very little peripheral sclerosis, no evidence of renal impairment and no edema or other evidence of cardiac failure. The basal metabolic rates on successive days were 25 and 22 per cent above the calculated normal.

The diagnosis was hypertension, thyrotoxicosis (mild), early vascular disease.

6 F B, a white male aged 40 years, complained of weakness and shortness of breath for one month previous to admission. The blood pressure was known to have been low for one month. On examination he was fairly well nourished and developed. There was some increased pigmentation of the exposed parts of the skin. There was no anemia. The cardiac dulness was not increased, the sounds were of poor quality, but there was no evidence of congestive heart failure. Adrenalin and extract of the whole gland produced no improvement and the patient died twenty days after admission. Post-mortem examination showed extreme atrophy of the adrenals.

The diagnosis was Addison's disease.

7 C B, a white male aged 24 years, complained of abdominal cramps and weakness for one year, with a loss of 36 pounds in 8 months. On examination the patient was found to be emaciated and rather pale with pigmentation suggesting Addison's disease. The cardiac dullness was 3 cm. to the right, 6 cm. to the left and the supra-cardiac dullness was 4 cm. There were a few moist râles in both lung apices. There was some tenderness and resistance in the lower right quadrant of the abdomen. The red blood cells numbered 4,510,000 per cm. The hemoglobin was 63 per cent.

The diagnosis was bilateral pulmonary tuberculosis, tuberculosis of the cecum, and Addison's disease (?)

8 W G, was a normal, healthy medical student 23 years of age.

9 M H., was a member of the hospital staff, 29 years of age and quite well

#### BIBLIOGRAPHY

- 1 O'Hare, James P, Calhoun, A. W, and Altnow, Hugo O, J Am. Med. Assoc., 1928, xc, 1436 The Etiology of Chronic Myocarditis
- 2 Evans, C Lovatt, J Physiol, 1918, lii, 6 The Velocity Factor in Cardiac Work
- 3 Blumgart, H. L, and Weiss, Soma, J Clin Invest., 1927, iv, 173 Studies on the Velocity of Blood Flow IV The Velocity of Blood Flow and Its Relation to Other Aspects of the Circulation in Patients with Arteriosclerosis and in Patients with Arterial Hypertension.
- 4 Plesch, Johann, Ztschr f Exper Pathol u Therap, 1909, vi, 380 Hämodynamische Studien
- 5 Liljestrand, G, and Stenström, N, Acta med. Scandinav, 1925, lxxii, 142 Clinical Studies on the Work of the Heart during Rest. III Blood Flow in Cases of Increased Arterial Pressure with Observations on the Influence of Pregnancy on the Blood Flow
- 6 Hayasaka, Enaji, Tohoku J Exper Med, 1927, ix, 401 On the Minute Volume of the Heart in Hypertension.
- 7 Krogh, A., and Lindhard, J, Skandinav Archiv f Physiol, 1912, xxvii, 100 Measurements of the Blood Flow through the Lungs of Man
- 8 Redfield, Alfred C, Bock, Arlie V, and Meakins, J C, J Physiol, 1922, lvi, 76 The Measurement of the Tension of Oxygen and Carbon Dioxide in the Blood of the Pulmonary Artery in Man.
- 9 Burwell, C Sidney, and Robinson, G Canby, J Clin Invest, 1924, i, 87 The Gaseous Content of the Blood and the Output of the Heart in Normal Resting Adults.
- 10 Field, H Jr, Bock, A V, Gildea, E V, and Lathrop, F L, J Clin Invest., 1924, i, 65 The Rate of the Circulation of the Blood in Normal Resting Individuals.

- 11 Burwell, C Sidney, Neighbors, DeWitt, and Regen, Eugene M , J Clin Invest , 1927, v, 125 The Effect of Digitalis upon the Output of the Heart in Normal Man
- 12 Holman, Emile, and Beck, Claude S , J Clin Invest , 1926, iii, 283 The Physiological Response of the Circulatory System to Experimental Alterations III The Effect of Aortic and Pulmonic Stenoses
- 13 Blalock, Alfred, Arch Surg , 1927, xv, 762 Mechanism and Treatment of Experimental Shock I Shock Following Hemorrhage
- 14 Erlanger, J , and Hooker, D R , Johns Hopkins Hosp Reports, 1904, vii, 145 An Experimental Study of Blood Pressure and of Pulse Pressure in Man
- 16 Rosen, I T , and White, H L , Am J Physiol , 1926, lxxviii, 168 The Relation of Pulse Pressure to Stroke Volume
- 17 Wiggers, Carl J , Modern Aspects of the Circulation in Health and Disease, Philadelphia, 1923, 2nd ed p 366

# THE EFFECT OF THE DIGITALIS BODIES ON THE VELOCITY OF BLOOD FLOW THROUGH THE LUNGS AND ON OTHER ASPECTS OF THE CIRCULATION A STUDY OF NORMAL SUBJECTS AND PATIENTS WITH CARDIOVASCULAR DISEASE<sup>1</sup>

BY SOMIA WEISS AND HERRMANN L. BLUMGART

*(From the Thorndike Memorial Laboratory of the Boston City Hospital, and the Department of Medicine, Harvard Medical School Boston)*

(Received for publication October 4, 1928)

Knowledge of the efficiency of the circulation in man before and after the administration of therapeutic measures is essential to establish a rational basis for the treatment of circulatory failure. Circulatory measurements in normal subjects and in patients before and after the administration of digitalis bodies are few. Burwell, Neighbors, and Regen (1) studied the changes in the minute volume output of the heart after the administration of large therapeutic doses of digitalis powder to four normal subjects. Following the administration of digitalis they observed, by the method of Field, Bock, Gildea, and Lathrop (2), no increase in the cardiac output per minute. On the contrary there was a slight but definite reduction of blood flow. The decrease in blood flow, according to these authors, could not be explained by the normal variation in the volume of blood expelled from the left ventricle per minute, or by technical error. In applying their results to patients with circulatory failure, they caution, however, that "If the same pharmacological effect upon heart muscle can produce different effects upon cardiac output in different states of heart muscle, it is then unsafe to apply our conclusions directly to an analysis of the effect of digitalis upon the cardiac output of patients suffering from heart failure." Eppinger, Papp, and Schwarz (3) observed a reduction in the amount of blood expelled from the left

<sup>1</sup> This investigation was aided by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases

ventricle of one patient who was suffering from circulatory failure. No significance can be attached to this finding for it is well recognized that the method they used does not give reliable results in patients with circulatory failure. Cohn and Stewart (4) studied the effect of digitalis by means of moving x-ray films, observing the change in the height of the left ventricular excursion of the heart. The four patients studied were young adults with symptoms and signs of rather mild circulatory failure. Two of the patients showed auricular fibrillation, the other two regular rhythm. In all four patients a significant increase in the height of the left ventricular excursion was seen, when the patients were under the effect of digitalis. An increase in the ventricular excursion, under such conditions, does not necessarily indicate, but is suggestive of increased output of the heart, provided that there is no corresponding decrease in rate, and provided there is no significant change in the size of the heart. Recently Hochrein and Meier (5), applying Bromser's method on man, noted that, following the administration of strophanthin, patients suffering from circulatory failure showed a decrease between the peaks of the pulse and pulse velocity curves simultaneously taken. This indicates that, following the administration of therapeutic doses of strophanthin, the blood flow through the radial arteries increased. Kinnmonth (6) using Henderson's ethyl iodid method observed that the circulation rate may be increased, decreased, or unaffected by digitalis in patients with diseased circulatory system. These variations in response to giving digitalis occurred not only in different patients, but in the same patient at different times. An increase in the circulation rate was more apt to occur in patients with low circulatory rate and with signs of circulatory failure. In patients with heart disease, but with no reduction of the circulatory rate, an increase occurred less frequently and the circulation rate was either unaltered or decreased. It should be recalled that the value of the ethyl iodid method in the form applied by Kinnmonth has been adversely criticised.

In view of these discrepant results, and the questionable reliability of the methods applied by some of the investigators, one is forced to conclude that the effect of digitalis on the blood flow in man and in patients with circulatory failure remains unknown.

Because preceding studies (7) have shown that the velocity of blood flow is a fundamental and characteristic aspect of the circulation both in health and in disease, and because a reliable method for the measurement of the velocity of blood flow has become available, we have investigated the effect of digitalis on the velocity of blood flow in normal subjects as well as in patients with cardiovascular disease

## METHODS

The methods employed in this investigation were similar to those used in previous studies (8). Simultaneously with the measurement of the velocity of the blood flow through the lungs, the velocity of venous blood flow from the right elbow to the right auricle was measured. The venous pressure was estimated by the method of Montz and Tabora (9), and the vital capacity of the lungs with a Collins spirometer. All measurements were performed after more than 12 hours of eating the last meal.

## RESULTS

### *A In normal subjects*

To ascertain the effect of the digitalis bodies on the velocity of blood flow of normal human beings, eight volunteer male subjects between the ages of 24 and 46 were studied (table 1). To observe the immediate effect of the administration of digitalis bodies, four of the subjects received strophanthin intravenously. The physiological activity of the specimen used was 0.14 mgm per cat unit, as estimated by the assay of Hatcher and Brody (10). The velocity of the blood flow and other aspects of the circulation were measured before and again after the administration of the strophanthin. In subjects 4, 6, and 8 the measurement of velocity was repeated three hours after injection of the drug, while in subjects 3 and 5 the tests were repeated six hours after the administration of strophanthin. Subjects 1, 6, and 7 received large therapeutic doses of tincture of digitalis by mouth. The physiological potency of the tincture used corresponded to 1 cat unit per 1 cc. Subjects 1 and 6 received an initial dose of 50 per cent of the total dose at once, followed by two doses

TABLE 1  
Effect of digitalis on the velocity of pulmonary blood flow of normal subjects

| Number | Name | Age | Date                         | Heart rate per minute | Arm to heart circulation time |                 | Pulmonary circulation time |                 | Medication     | Method of administration |
|--------|------|-----|------------------------------|-----------------------|-------------------------------|-----------------|----------------------------|-----------------|----------------|--------------------------|
|        |      |     |                              |                       | Seconds                       | Change, seconds | Seconds                    | Change, seconds |                |                          |
| 1      | V F  | 46  | 1927<br>March 16<br>March 17 | 70<br>58              | 6 5<br>10 0                   | +3 5            | 14 0<br>17 5               | +3 5            | Tr dig 19 cc   | Mouth                    |
| 2      | W II | 35  | January 19<br>January 19     | 103<br>91             | 5 5<br>6 0                    | +0 5            | 8 0<br>8 5                 | +0 5            | Stroph 0.9 mgm | Vein                     |
| 3      | J M  | 35  | January 18<br>January 18     | 83<br>93              | 5 0<br>5 5                    | +0 5            | 8 0<br>8 0                 | 0 0             | Stroph 0.7 mgm | Vein                     |
| 4      | J D  | 24  | January 18<br>January 19     | 79<br>83              | 7 5<br>12 0                   | +4 5            | 11 5<br>11 5               | 0 0             | Stroph 0.9 mgm | Vein                     |
| 5      | C W  | 35  | January 19<br>January 19     | 112<br>97             | 6 0<br>8 0                    | +2 0            | 8 5<br>8 0                 | -0 5            | Stroph 0.7 mgm | Vein                     |
| 6      | I D  | 41  | January 16<br>January 17     | 55<br>57              | 13 0<br>8 5                   | +4 5            | 12 0<br>11 0               | -1 0            | Tr dig 17 cc   | Mouth                    |
| 7      | L I  | 21  | January 6<br>January 18      | 59<br>54              | 5 0<br>5 5                    | +0 5            | 8 5<br>11 0                | -2 5            | Tr dig 20 cc   | Mouth                    |
| 8      | M M  | 35  | January 19<br>January 19     | 111<br>100            | 5 0<br>4 0                    | -1 0            | 10 0<br>7 5                | -2 5            | Stroph 0.9 mgm | Vein                     |

of 25 per cent of the total calculated dose at six hour intervals. The circulatory tests were performed six hours after the last dose. Subject 7 received tincture of digitalis in the same manner as just described. Circulatory measurements in the case of this individual were performed 12 hours after the administration of the last dose, when this normal person showed electrocardiographic evidence of the effect of digitalis. The same measurements were repeated twelve days later, when the dose of digitalis previously administered had probably been eliminated. Toxic reactions except for nausea and vomiting in subjects 1 and 7 were not observed. Table 1 illustrates that although there was a variation in dosage and method of administration of the digitalis bodies, the velocity of the blood flow showed no significant alteration. The average difference between the circulation time of the venous blood flow from the cubital vein to the right side of the heart was an increase of 1.9 seconds, the average difference between the pulmonary circulation times of the eight normal subjects studied was a decrease of 0.3 second. The maximum variation before and after the administration of the digitalis bodies was an increase of 4.5 seconds in the venous circulation time, and an increase of 3.5 seconds in the pulmonary circulation time. We have called attention to the fact that the velocity of the venous blood flow from the arm to the right side of the heart shows greater spontaneous variations both in normal individuals and in patients with circulatory failure, than the velocity of the blood flow in the pulmonary circuit (8). The arterial and venous blood pressures, as well as the vital capacities of the lungs of the eight subjects studied, showed no changes which could be attributed to a digitalis effect.

#### *B In patients with cardiovascular disease*

The results of the investigation of the effect of the digitalis bodies on the velocity of blood flow of patients suffering from cardiovascular disease is presented in table 2. A summary of the clinical observations concerning the cardiovascular system of the 14 patients is appended at the end of this publication. It is evident that both the abnormality of the cardiovascular system and the degree of circulatory failure varied considerably. Patients 7, 8, 9, 10, 11 and 14 exhibited only symptoms and signs of circulatory failure on exertion,

TABLE 2

*Effect of digitalis bodies on the velocity of pulmonary of blood flow of patients suffering from cardiovascular diseases*

| Num-<br>ber | Name | Age | Diagnosis  | Before<br>After | Date        | Pulse | Venous<br>pressure<br><br>cm. H <sub>2</sub> O | Vital<br>capac-<br>ity per<br>square<br>meter | Arm to heart<br>circulation<br>time |                    | Pulmonary<br>circulation<br>time |                    | Medication    | Method of<br>adminis-<br>tration |
|-------------|------|-----|--|-----------------|-------------|-------|--|---|-------------------------------------|--------------------|----------------------------------|--------------------|---------------|----------------------------------|
|             |      |     |  |                 |             |       |  |   | Sec-<br>onds                        | Change,<br>seconds | Sec-<br>onds                     | Change,<br>seconds |               |                                  |
| 1           | J M  | 62  | Myocardial degeneration                                  | Before          | March 6     | 100   | +9 0   | 800   | 22 0                                | -6 0               | 45 0                             | -13 0              | Tr dig 16 cc. | Mouth                            |
|             |      |     |  | After           | March 8     | 72    | +6 5   | 900   | 16 0                                |                    | 32 0                             |                    |               |                                  |
| 2           | J C  | 57  | Myocardial degeneration                                  | Before          | February 23 | 84    | +5 5   | 1,361   | 13 0                                | 0 0                | 16 5                             | -8 0               | Tr dig 22 cc  | Mouth                            |
|             |      |     |  | After           | February 23 | 87    | +4 0   | 1,951   | 13 0                                |                    | 8 5                              |                    |               |                                  |
| 3           | P T  | 63  | Arterial hypertension, myo-<br>cardial degeneration      | Before          | May 12      | 81    | +4 5   | 1,790   | 8 0                                 | 0 0                | 21 0                             | -7 0               | Tr dig 16 cc  | Mouth                            |
|             |      |     |  | After           | May 14      | 73    | +2 0   | 1,790   | 8 0                                 |                    | 14 0                             |                    |               |                                  |
| 4           | C B  | 41  | Arterial hypertension, myo-<br>cardial degeneration      | Before          | May 18      | 118   | +21 0  | 650   | 22 0                                | -15 5              | 22 0                             | -6 0               | Tr dig 20 cc  | Mouth                            |
|             |      |     |  | After           | May 21      | 82    | +6 5   |   | 6 5                                 |                    | 16 0                             |                    |               |                                  |
| 5           | T J  | 52  | Generalized arteriosclerosis,<br>myocardial degeneration | Before          | February 15 | 74    | +4 5   | 1,960   | 10 0                                | +2 0               | 22 0                             | -5 0               | Tr dig 18 cc  | Mouth                            |
|             |      |     |  | After           | February 17 | 68    | +2 0   | 1,960   | 12 0                                |                    | 17 0                             |                    |               |                                  |
| 6           | P M  | 50  | Myocardial degeneration                                  | Before          | May 12      | 64    | +3 5   | 964   | 11 0                                | +4 0               | 14 5                             | -4 5               | Tr dig 21 cc  | Mouth                            |
|             |      |     |  | After           | May 16      | 58    | -1 0   | 1,384   | 15 0                                |                    | 10 0                             |                    |               |                                  |
| 7           | A S  | 74  | Arterial hypertension, myo-<br>cardial degeneration      | Before          | March 6     | 78    | +5 5   | 2,330   | 9 0                                 | +5 0               | 24 5                             | -4 5               | Tr dig 16 cc  | Mouth                            |
|             |      |     |  | After           | March 8     | 66    | +2 0   | 2,610   | 14 0                                |                    | 20 0                             |                    |               |                                  |

|    |      |    |   |                          |   |                |                             |                      |                      |                      |                 |       |
|----|------|----|---|--------------------------|---|----------------|-----------------------------|----------------------|----------------------|----------------------|-----------------|-------|
| 8  | P P  | 68 | Auricular fibrillation, arterio-sclerosis                         | Before<br>After          | February 13<br>February 15                | 88<br>75       | +5 52 230<br>+5 52 230      | 10 0<br>8 0          | -2 0<br>12 0         | 15 5<br>-3 5         | Stroph 10 mgm.  | Vein  |
| 9  | W M  | 65 | Syphilitic heart disease, aortic insufficiency                    | Before<br>After          | March 8<br>March 10                       | 72<br>74       | +2 02 000<br>+2 51,950      | 10 0<br>7 0          | -3 0<br>16 0         | 17 0<br>-1 0         | Tr dug 19 cc.   | Mouth |
| 10 | A. O | 68 | Arterial hypertension, myocardial degeneration                    | Before<br>After          | January 6<br>January 6                    | 78<br>60       | +7 01 790<br>+10 51 440     | 10 0<br>8 5          | -1 5<br>15 0         | 13 0<br>+2 0         | Stroph. 0.8 mgm | Vein  |
| 11 | W P  | 39 | Syphilitic heart disease, aortic insufficiency                    | Before<br>After          | March 8<br>March 10                       | 84<br>72       | +1 02 130<br>+1 02,090      | 5 0<br>5 5           | -12 0<br>+0 5        | 12 0<br>14 0         | Tr dig 17 cc    | Mouth |
| 12 | D K  | 61 | Myocardial degeneration, auricular fibrillation                   | Before<br>After<br>After | February 21<br>February 23<br>February 28 | 80<br>64<br>60 | +4 01 580<br>1 580<br>1,790 | 14 0<br>15 0<br>14 0 | -20 0<br>25 0<br>0 0 | 20 0<br>25 0<br>21 0 | Tr dig 20 cc    | Mouth |
| 13 | L. S | 39 | Rheumatic heart disease, mitral stenosis and insufficiency        | Before<br>After          | March 16<br>March 17                      | 62<br>55       | -5 02 730<br>-4 52,730      | 13 5<br>10 0         | -3 5<br>22 0         | 15 0<br>+7 0         | Tr dig. 14 cc.  | Mouth |
| 14 | S C. | 26 | Rheumatic heart disease, pericarditis with auricular fibrillation | Before<br>After          | December 7<br>December 7                  | 76<br>62       | +10 01,690<br>+8 51,590     | 9 5<br>15 5          | -6 0<br>26 0         | 19 0<br>+7 0         | Stroph 0.8 mgm. | Vein  |

while patients 1, 2, 3, 4, 5, 6, 12 and 13 showed signs of congestive failure of the circulation even when at rest. Patients 8, 12 and 14 exhibited fibrillation of the auricles, while in the other patients the rhythm was regular. In order to differentiate between the effect of rest and of digitalis, all patients were kept in bed for from 8 to 30 days, until clinical observations, electrocardiographic measurements, and tests of the vital capacity indicated no further improvement. Tested tincture of digitalis or strophanthin was then administered in large therapeutic doses. The effects of full digitalization were evidenced within 24 hours after beginning the administration of the drug. Circulatory measurements were then repeated within 24 hours after the administration of the last dose in all cases with the exception of patient 6, in whom the measurements were repeated 2 days after the last dose. All patients showed one or more effects of digitalization, such as slowing of the pulse rate, inversion of the T waves in the leads of the electrocardiographic tracings, or the appearance of nausea. Several patients showed several of these effects. As the main interest in this study was to observe the effect of the digitalis bodies on the pulmonary blood flow, which is closely related to the state of the entire body, the data for the patients are grouped in table 2 according to the degree of change in the pulmonary circulation rate following the administration of tincture of digitalis or strophanthin. Patient 1 showed the most marked decrease, and patient 14 the most marked increase of the pulmonary circulation time. The results indicate that only three patients (12, 13, and 14) of the fourteen patients studied showed a definite slowing of the pulmonary blood flow. In one of these three patients (number 12) six days after the beginning of the administration of digitalis the velocity was the same as before. In the other eleven patients the velocity of pulmonary blood flow was either unaltered or there was a definite increase as the result of the administration of the digitalis bodies. Accepting 3.5 seconds as the maximum normal variation, as was shown by the measurements on normal subjects, seven of the fourteen patients showed a definite increase above normal. The average increase in these seven patients was 6.9 seconds. The findings indicate no relationship between the changes in the velocity of pulmonary blood flow and that of the venous blood flow from the arm

to the heart. The vital capacities of the lungs showed no definite changes after the administration of digitalis. The venous pressure became definitely lowered in patients 1 and 4. No relationship could be established between the severity of circulatory failure and changes in velocity. The pulse rate was lower in all the patients with the exception of patients 2 and 9. The average slowing of the cardiac rate was 12 per minute (from 81 beats to 69). Definite correlation could not be established between the degree of circulatory failure and change in the pulmonary circulation time, although six of the seven patients who reacted with a definite increase in velocity showed symptoms and signs of congestive failure at the time of test. These patients were numbers 1, 2, 3, 4, 5 and 6. It is of interest to note that two of the three patients who exhibited fibrillation of the auricles showed definite prolongation of the pulmonary circulation time after the administration of large therapeutic doses of tincture of digitalis. These were patients 12 and 14. Patient 8, however, showed a decrease of 3.5 seconds.

#### DISCUSSION

The results of this study indicate that tincture of digitalis and strophanthin, and thus presumably other digitalis bodies, when administered to normal subjects under the conditions described, exert no appreciable change on the velocity of the pulmonary blood flow. When digitalis and strophanthin were administered to patients with cardiovascular disease the velocity of flow in the pulmonary circuit became distinctly slower in three patients, while in the rest of the eleven patients there was either no change, or a definite increase. The observations also indicate that following the administration of digitalis changes are greater in cardiac patients than in normal subjects.

Our observations in the past (7) (11) demonstrated that in circulatory failure there is slowing of the velocity of blood flow. Although slowing is not always proportional to the degree of clinical decompensation, with clinical improvement there is nevertheless generally an increase in the velocity of blood flow. The cardiac output of the heart in circulatory failure is not definitely known at present because of technical difficulties connected with the measurements. The conception expressed by certain investigators that with circulatory failure

there is an increase in the cardiac output could be reconciled with the findings of slowing of the pulmonary velocity only if one assumes that in cardiac failure there is considerable increase in the available cross sectional area of the vascular bed. At present experimental or clinical data are not available which throw light on this aspect of the problem. Similarly, no direct reliable observation on patients with circulatory failure is available which supports the theory that the beneficial effect of digitalis on the circulation manifests itself in a sedative action, decreasing the minute volume output of the heart. Our finding that seven out of fourteen patients with circulatory failure showed an increase in the velocity of blood flow in the pulmonary circuit following the administration of digitalis, is in harmony with other numerous observations on patients, in whom during their stay in hospital repeated measurements of the velocity of blood flow were taken. According to these observations, with improvement in the general clinical condition of the patient, the velocity of blood flow and the vital capacity increased and the venous pressure became lower.

The change in velocity of the blood flow following the administration of digitalis is therefore the same as the change which follows other therapeutic measures, which are beneficial in the treatment of circulatory failure. The observation that those patients who showed clinical improvement after the administration of digitalis bodies showed likewise an increase in the velocity of blood flow is of greater significance in the analysis of digitalis effect in patients, than finding no change or slowing. To condemn the use of digitalis because it fails to improve the circulation in a few patients is as irrational as giving up arsenicals because they do not always better patients with syphilis. It is often observed clinically that, depending probably on structural and physiological changes in the heart, a stage is reached when digitalis fails to benefit. In rare instances the symptoms and signs of circulatory failure may become definitely worse after the administration of digitalis and improvement may follow the omission of the drug. Our observations suggest that the digitalis bodies do not induce uniform changes in the velocity of blood flow of patients suffering from circulatory failure. This is in harmony with the findings of others on the effect of digitalis on the minute volume output of the heart, as well as on the cardiac rate, conducting

system, and vital capacity. If we consider that the degree as well as the nature of pathological changes in the structures through which digitalis influences the circulation vary markedly from patient to patient, it is questionable whether a uniform change in circulatory measurements after the administration of digitalis bodies can be expected.

The fact that seven patients showed definite increase in the velocity of pulmonary blood flow indicates that the digitalis bodies are useful agents in the treatment of circulatory failure. Since the relatively direct approach to the problem of the action of digitalis in patients with circulatory failure entails a number of variables it can be readily understood how hazardous is the application of observations gained in studying animals, or normal men.

#### SUMMARY AND CONCLUSIONS

1 Strophanthin and tincture of digitalis were administered intravenously and by mouth respectively to eight normal persons. Their effect on the velocity of pulmonary and peripheral venous blood flow, on the vital capacities of the lungs and arterial and venous pressures, were observed. Amounts of these drugs corresponding to large therapeutic doses failed to change appreciably the velocity of the pulmonary blood flow and the other above mentioned aspects of the circulation in the normal subjects.

2 When strophanthin, or tincture of digitalis in large therapeutic doses was administered to 14 patients suffering from cardiovascular disease, the velocity of the pulmonary blood flow became increased in seven, was unaltered in four, while in three patients it was definitely decreased.

3 Although the average pulse rate in seven patients showed a reduction of 14 beats per minute, the pulmonary circulation time showed an average reduction of 6.9 seconds, which corresponds to an increase of 30 per cent in the velocity of pulmonary blood flow.

4 The velocity of blood flow in the pulmonary circuit is decreased in patients with circulatory failure. With clinical signs of improvement due to the administration of digitalis or to rest the velocity of this blood flow increases, although the degree of the patient's improvement and the change in velocity may not be parallel.

5 Patients with symptoms and signs of congestive failure, even when at rest, have had a greater tendency to show definite increase in the velocity of blood flow in the pulmonary circuit following the administration of digitalis than patients who were compensated at rest

6 The studies presented help to provide a rational basis for the therapeutic administration of digitalis to patients suffering from circulatory failure

#### ABSTRACTS OF HISTORIES AND PHYSICAL EXAMINATIONS OF PATIENTS STUDIED

1 J M, aged 62, complained of increasing dyspnea of one and a half years' duration For three months he had been unable to walk because of marked weakness He noticed marked swelling of the abdomen and legs He was cyanotic and dyspneic The heart was moderately enlarged The sounds were distant The cardiac rate per minute was 130 to 140 The rhythm was regular The blood pressure was 120 mm systolic and 50 mm diastolic of mercury Over the base of the lungs moist bubbling râles were heard The abdomen was distended The liver edge was felt 5 cm below the costal margin Marked pitting edema was noted over the buttocks and legs The condition of the patient was unaltered at the time of the administration of digitalis The diagnosis of myocardial degeneration was made

2 J C, aged 57, complained of shortness of breath and inability to work for one and a half years He noticed swelling of the feet following exertion The heart was normal in size The cardiac rate was rapid The sounds were distant No murmurs were heard At time of the administration of the digitalis bodies the patient was slightly dyspneic The diagnosis of myocardial degeneration was made

3 P F, aged 63, was suffering from nocturnal attacks of dyspnea During the last two years he had become weak and unable to work Occasionally he noticed swelling of the ankles On physical examination the heart was enlarged, the greatest diameter being 14 cm, and the aortic second sound was markedly accentuated The rhythm was regular Slight pitting edema was noticed over the ankles The blood pressure was 210 mm of systolic and 120 mm diastolic of mercury The patient's condition was unchanged at the time of the administration of digitalis The diagnosis of arterial hypertension was made

4 C B, aged 41, complained of shortness of breath and weakness for one year He noticed gradually increasing swelling of the ankles and legs during the six months previous to his entry to hospital He was unable to walk during the same period On physical examination he showed marked dyspnea and orthopnea The lips were cyanotic The heart was large The greatest transverse diameter was 13 cm The heart sounds were normal, and no murmur was heard The

rate was rapid, 110 to 130. The rhythm was regular. There was marked swelling of the abdomen with a suggestive fluid wave. The liver area was tender. The legs were swollen with marked pitting edema. The blood pressure was 195 mm systolic and 160 mm diastolic of mercury. The patient's condition did not improve under rest in bed, and the symptoms and signs of circulatory failure were unchanged at the time of administration of digitalis. The diagnosis of arterial hypertension was made.

5 Th J, aged 52, had been well until fourteen months previous to his entrance into the hospital, when he noticed cramp like pain starting in the precordium and radiating to the epigastrium. This attack was repeated several times, he became very dyspneic on such occasions. During the last four weeks he noticed marked swelling of the ankles, which disappeared after rest. During the last two weeks he also coughed a great deal and has had several attacks of palpitation. The apex impulse was observed in the sixth interspace. The first sound at apex was short and merged into a soft systolic murmur. The second sound was reduplicated all over the precordium. The cardiac rhythm was regular. The peripheral vessels were sclerosed. A moderate degree of edema was observed over both ankles at the time of entrance into the hospital. The blood pressure was 140 mm systolic and 100 mm diastolic of mercury. At the time of test the general condition of the patient was the same, except that no edema was observed. The diagnosis of arteriosclerotic heart disease was made.

6 P M, aged 50, noticed one month previous to his entry increasing shortness of breath, cough, swelling of the legs and abdomen. He was unable to work. On physical examination he was markedly orthopneic. The apex of the heart was not felt. The heart sounds were distant. The cardiac rate was rapid and the rhythm regular. The blood pressure was 155 mm systolic and 80 mm diastolic of mercury. The right side of the chest posteriorly was flat on percussion and the breath sounds were suppressed. Moist bubbling rales were heard over the base on both sides. The abdomen was tender over the liver area and it was large. The legs were markedly swollen. The condition was essentially unchanged at the time of administration of digitalis. The diagnosis of myocardial degeneration was made.

7 A S, aged 74, complained of frequent attacks of precordial pain, associated with dyspnea. No other evidence of circulatory failure was obtained from the history. On physical examination some tortuosity and sclerosis of the retinal vessels were observed. The lips were cyanotic. The heart was of normal size. The heart sounds were weak but regular. The peripheral vessels were markedly thickened. The blood pressure was 190 mm systolic and 95 mm diastolic of mercury. Numerous moist rales were heard over the bases of the lungs. The diagnosis of arteriosclerotic heart disease and arterial hypertension was made.

8 P P, aged 68, complained of shortness of breath and weakness. He had noticed swelling of the feet frequently after work. On physical examination the heart was found enlarged. The maximum of the transverse diameter was 13 cm.

The sounds were rather distant. The rhythm was totally irregular. The peripheral vessels were moderately sclerosed. The blood pressure was 120 mm systolic and 70 mm diastolic of mercury. No edema was noted. The condition of the patient at the time of test was essentially unchanged. The diagnosis of arteriosclerosis and auricular fibrillation was made.

9 W M, aged 65, had had shortness of breath during the last six months previous to his admission. Two weeks previous to entry he noticed sharp abdominal pains which became aggravated after meals. He never observed swelling of the ankles, or of the abdomen. Physical examination showed an enlarged heart with a maximum transverse diameter of 14 cm. The heart sounds were of good quality and the pulmonary second sound was accentuated. Over the third right intercostal space a rough systolic murmur and a prolonged blowing diastolic murmur were heard. The peripheral vessels were moderately thickened. The pulses were full and bounding. Blood pressure was 196 mm systolic and 50 mm diastolic of mercury. No pitting edema or other signs of congestive cardiac failure observed. The Wassermann test of the blood was positive. The diagnosis of syphilitic heart disease with aortic insufficiency was made.

10 A O, aged 68, gave no history of circulatory failure. He had been told that his blood pressure was high. The heart was slightly enlarged. The greatest transverse diameter was 12 cm on percussion. The aortic second sound was markedly accentuated. The blood pressure was 228 mm systolic and 112 mm of diastolic. The diagnosis of arterial hypertension was made.

11 W P, aged 39, suffered from attacks of precordial pain of two years' duration. During the last three weeks he had been suffering from shortness of breath which was worse at night. He also coughed a great deal during the same period. He had had no edema of the ankles despite the fact that he walked much. Physical examination showed marked pulsation of the vessels of the neck, over which a systolic murmur was heard. The apex impulse of the heart was felt in the sixth intercostal space in the anterior axillary line. The heart was enlarged downward and outward. The sounds were of good quality, with regular rhythm. Loud to and fro murmur was heard over the precordium with the maximum intensity over the aortic area. The radial pulses were of the Corrigan type. The blood pressure was 164 mm systolic and 40 mm diastolic of mercury. The Wassermann and Kahn tests of the blood were positive. The patient's condition was the same as just described at the time of the test. The diagnosis of syphilitic heart disease with aortic insufficiency was made.

12 D K, aged 61, had had shortness of breath on exertion for one year. One month previous to his admission he became so weak that he could walk short distances only. No history of edema was obtained. The complaints were essentially the same as at the time of test. On physical examination the cardiac impulse was rather diffuse. The maximum transverse diameter was 13 cm. The sounds were of fairly good quality. At the apex a soft systolic murmur was heard. The rhythm was totally irregular. The peripheral vessels showed

moderate thickening. The blood pressure was 110 mm systolic and 70 mm diastolic of mercury. The liver edge was 3 cm below the costal border. The diagnosis of arteriosclerotic heart disease and auricular fibrillation was made.

13 L S, aged 39, was suffering from severe attacks of rheumatic fever at the age of 18. For one year he had noticed shortness of breath on exertion, as well as occasional swelling of the ankles. He was slightly dyspneic. The heart was slightly enlarged, the greatest transverse diameter being 12 cm. The first sound over the apex was loud. Over the same area a systolic and diastolic murmur was heard. The rhythm was regular. The liver was not palpated. Slight pitting edema over the ankles was noted. The blood pressure was 108 mm systolic and 60 mm diastolic of mercury. Under rest the patient improved and at the time of administration of digitalis he was not dyspneic and no edema was present. The diagnosis of rheumatic heart disease with mitral stenosis and insufficiency was made.

14 S C, aged 26, complained of shortness of breath. He had had rheumatic fever in childhood but had been well until nine years previously, when after pneumonia, he developed moderate shortness of breath for eight months. During the ten months before entry he experienced slight precordial pain on exertion with shortness of breath and palpitation, which gradually increased in severity forcing him to enter the hospital. Three days before admission, pitting edema was observed over lower legs. After admission to the hospital, on rest in bed and on taking digitalis, he showed moderate improvement. Physical examination showed blowing systolic and diastolic murmurs over aortic and mitral areas. The liver was not palpable. There was no edema of the legs. A few moist râles were heard over the left base. Following rest in hospital patient returned home but was compelled to re-enter hospital because of exacerbation of symptoms. At time of administration of strophanthin patient was up and about the ward. There was no dyspnea or orthopnea. Physical examination showed no râles over chest and no edema. The liver was not palpable, and the heart was as noted above. The diagnosis was rheumatic pericarditis, auricular fibrillation, aortic stenosis and insufficiency, mitral stenosis and insufficiency.

#### BIBLIOGRAPHY

- 1 Burwell, C S, Neighbors, D W, and Regen, E M, J Clin Invest., 1927, v, 125. The Effect of Digitalis upon the Output of the Heart in Normal Man.
- 2 Field, H Jr, Bock, A. V, Gildea, E F, and Lathrop, F L, J Clin. Invest., 1924, 1, 65. The Rate of the Circulation of the Blood in Normal Resting Individuals.
- 3 Eppinger, H, Von Papp, L, and Schwarz, H, Über das Asthma Cardiale, Versuch zu einer peripheren Kreislaufpathologie. Julius Springer, Berlin, 1924, p 191.

- 4 Cohn, A E , and Stewart, H J , J Clin Invest , 1924, 1, 97 Evidence that Digitalis Influences Contraction of the Heart in Man
- 5 Hochrein, M , and Meier, R , Munchen Med Wchnschr , 1927, lxxiv, 1995 Ueber neuere Methoden Zur Bestimmung der Arterellen Blutgeschwindigkeit
- 6 Kinnmonth, J G , Quart J Med , 1928, xxi, 277 The Circulation Rate in Some Pathological States, with Observations on the Effect of Digitalis
- 7 Blumgart, H L , and Weiss, Soma, J Clin Invest , 1927, iv, 199 Studies on the Velocity of Blood Flow V The Physiological and the Pathological Significance of the Velocity of Blood Flow
- 8 Blumgart, H L , and Weiss, Soma, J Clin Invest , 1927, iv, 399 Studies on the Velocity of Blood Flow VII The Pulmonary Circulation Time in Normal Resting Individuals
- 9 Moritz, F , and Tabora, D V , Deutsche Arch f Klin Med , 1910, xcvi, 475 Ueber eine Methode, beim Menschen den Druck die Oberflächlichen Venen exakt zu bestimmen
- 10 Hatcher, R A , and Brody, J G , Am J Pharmacy, 1910, lxxxii, 360 The Biological Standardization of Drugs
- 11 Blumgart, H L , and Weiss, Soma, J Clin Invest , 1928, v, 343 Clinical Studies on the Velocity of Blood Flow IX The Pulmonary Circulation Time, the Velocity of Venous Blood Flow to the Heart, and Related Aspects of the Circulation in Patients with Cardiovascular Disease

# OBSERVATIONS ON PAROXYSMAL HEMOGLOBINURIA<sup>1</sup>

By G. M. MACKENZIE

WITH THE TECHNICAL ASSISTANCE OF EMILY FRÜHBAUER

(From the Medical Clinic of the Presbyterian Hospital, New York and the Medical Clinic of the Mary Imogene Bassett Hospital, Cooperstown, N. Y.)

(Received for publication October 23, 1928)

For the discovery of the reaction which reproduces in vitro what appears to be the essential feature of the mechanism of this disease we are indebted to Donath and Landsteiner (1). They showed that the serum of these patients contains an auto-hemolysin which unites with the patient's own red blood cells or with those of any other individual only at a low temperature, that when such a mixture is warmed to 37°C hemolysis occurs if complement be present. Complement is essential for the completion of the reaction. Rosenbach (2) had previously shown that all the phenomena of a typical paroxysm of the disease may be produced by immersion of the hands or feet of the patient in ice water.

During the past nine years I have had five of these patients under observation and this paper summarizes some of the serological, clinical and therapeutic results.

This auto-hemolysin has certain striking peculiarities. At body temperature it will not unite with the red blood cells. The highest temperatures at which union could be detected with the auto-hemolysins from three of the patients I have studied were respectively 12°C, 12°C and 10°C (protocol 1). Temperatures down to 4°C or 5°C, however, gave more complete union, shown by greater hemolysis when the mixture was subsequently warmed. In two of these patients hemolysis was greater if the red cells from the patient were used rather than those from another individual of the same blood group (protocol 2). In all five of these patients it has been possible to confirm the observation of Yorke and Macfie (3) that union is more effective

<sup>1</sup> Presented in abstract form at the meeting of the Association of American Physicians, May 2, 1928.

and hemolysis greater if the chilling is limited to five or ten minutes rather than the more usual period of thirty minutes (protocol 3) This is particularly interesting in view of what occurs when a patient spontaneously has a paroxysm The actual time during which the

# PROTOCOL 1

December 7, 1922 Patient L O Blood drawn from arm vein, one portion allowed to clot at room temperature, serum separated 30 minutes after venepuncture, serum in ice box over night. A second portion was taken in citrate and used for the preparation of the red cell suspension Control serum and red cell suspension prepared in the same way from a normal adult belonging to the same blood group

| Tube | Patient's serum unheated | Patient's red blood cells 5 per cent | Control serum unheated (Group A) | Control red blood cells 5 per cent (Group A) | Complement 1 10 (guinea pig) | NaCl 0.85 per cent | Kept at                       | Hemolysis |
|------|--------------------------|--------------------------------------|----------------------------------|--|------------------------------|--------------------|-------------------------------|-----------|
| 1    | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | 0°C 10 minutes, 37°C 2 hours  | ++        |
| 2    | 0 25                     |                                      |                                  | 0 1  | 0 1                          | 0 05               |                               | ++        |
| 3    | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | 4°C 10 minutes, 37°C 2 hours  | +(+)      |
| 4    | 0 25                     |                                      |                                  | 0 1  | 0 1                          | 0 05               |                               | ++        |
| 5    | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | 8°C 10 minutes, 37°C 2 hours  | +(+)      |
| 6    | 0 25                     |                                      |                                  | 0 1  | 0 1                          | 0 05               |                               | +(+)      |
| 7    | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | 12°C 10 minutes, 37°C 2 hours | +         |
| 8    | 0 25                     |                                      |                                  | 0 1  | 0 1                          | 0 05               |                               | +         |
| 9    | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | 16°C 10 minutes, 37°C 2 hours | 0         |
| 10   | 0 25                     |                                      |                                  | 0 1  | 0 1                          | 0 05               |                               | 0         |
| 11   |                          |                                      | 0 25                             | 0 1  | 0 1                          | 0 05               | 0°C 10 minutes, 37°C 2 hours  | 0         |
| 12   |                          | 0 1                                  | 0 25                             |  | 0 1                          | 0 05               | 0°C 10 minutes, 37°C 2 hours  | 0         |
| 13   | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | Not chilled, 37°C 2 hours     | 0         |
| 14   |                          |                                      | 0 25                             | 0 1  | 0 1                          | 0 05               | Not chilled, 37°C 2 hours     | 0         |
| 15   |                          |                                      |                                  | 0 1  | 0 1                          | 0 3                | 0°C 10 minutes, 37°C 2 hours  | 0         |
| 16   |                          | 0 1                                  |                                  |  | 0 1                          | 0 3                | 0°C 10 minutes, 37°C 2 hours  | 0         |

blood in superficial capillaries is exposed to lowered temperatures is obviously short It soon passes back to the higher temperatures of the interior of the body

These auto-hemolysins also have in some instances a surprising thermolability In one case 45°C for thirty minutes destroyed the auto-hemolysin so that the addition of fresh complement would not reacti-

vate it, in another case it was destroyed at 47.5°C. The extreme lability of this strange hemolysin is further shown by the fact that sometimes after one to three days in the ice box the serum will no longer cause lysis of the red cells. The hemolysin has disappeared (protocol 6). It has been shown repeatedly that the serum of par-

## PROTOCOL 2

January 2, 1925 Patient F V Blood drawn from arm vein one portion was allowed to clot at room temperature, serum separated as soon as clot had formed and test set up at once a second portion was citrated and used for the preparation of the red cell suspension. Control serum and red cell suspension obtained from a normal adult belonging to the same blood group as the patient.

| Tube | Patient's serum unheated | Patient's red blood cells 5 per cent | Control serum unbeated (Group O) | Control red blood cells 5 per cent (Group O) | Complement 1:10 (guinea pig) | NaCl 0.85 per cent | Kept at                      | Hemolysis |
|------|--------------------------|--------------------------------------|----------------------------------|--|------------------------------|--------------------|------------------------------|-----------|
| 1    | 0 3                      | 0 1                                  | α                                | α  | 0 1                          |                    | 0°C 10 minutes, 37°C 2 hours | ++        |
| 2    | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | 0°C 10 minutes, 37°C 2 hours | +(+)      |
| 3    | 0 2                      | 0 1                                  |                                  |  | 0 1                          | 0 1                | 0°C 10 minutes, 37°C 2 hours | +         |
| 4    | 0 1                      | 0 1                                  |                                  |  | 0 1                          | 0 2                | 0°C 10 minutes, 37°C 2 hours | 0         |
| 5    | 0 3                      |                                      |                                  | 0 1  | 0 1                          |                    | 0°C 10 minutes, 37°C 2 hours | +         |
| 6    | 0 25                     |                                      |                                  | 0 1  | 0 1                          | 0 05               | 0°C 10 minutes, 37°C 2 hours | 0         |
| 7    | 0 2                      |                                      |                                  | 0 1  | 0 1                          | 0 1                | 0°C 10 minutes, 37°C 2 hours | 0         |
| 8    | 0 1                      |                                      |                                  | 0 1  | 0 1                          | 0 2                | 0°C 10 minutes, 37°C 2 hours | 0         |
| 9    |                          | 0 1                                  | 0 25                             |  | 0 1                          | 0 05               | 0°C 10 minutes, 37°C 2 hours | 0         |
| 10   |                          |                                      | 0 25                             | 0 1  | 0 1                          | 0 05               | 0°C 10 minutes, 37°C 2 hours | 0         |
| 11   |                          | 0 1                                  |                                  |  | 0 1                          | 0 3                | 0°C 10 minutes, 37°C 2 hours | 0         |
| 12   |                          |                                      |                                  | 0 1  | 0 1                          | 0 3                | 0°C 10 minutes, 37°C 2 hours | 0         |
| 13   | 0 3                      | 0 1                                  |                                  |  | 0 1                          |                    | Not chilled, 37°C 2 hours    | 0         |
| 14   | 0 25                     | 0 1                                  |                                  |  | 0 1                          | 0 05               | Not chilled, 37°C 2 hours    | 0         |

oxysmal hemoglobinurics causes lysis not only of the patient's own red cells but also of the red cells of any other individual. I have made several attempts to separate by absorption experiments the auto-hemolysin from the iso-hemolysin (protocol 4). But in no case was one removed without also removing the other, hence they are probably identical.

Whether it is necessary to have complement present at the low temp-

perature is a question which has been answered in different ways by different observers (4, 5) So far as my observations go they indicate that complement may be added after the chilled serum and cells have been warmed to 37.5° and still have hemolysis occur, but that if alexin be present in the mixture at the low temperature, more complete hemolysis occurs (protocol 5)

### PROTOCOL 3

November 29, 1921 Patient L O Blood drawn from arm vein, one portion allowed to clot at room temperature, serum separated as soon as coagulation was complete and test set up immediately, a second portion was citrated and used for the preparation of the red cell suspension Control serum and red cell suspension obtained in the same way from a normal adult of the same blood group

| Tube | Patient's serum unheated | Patient's red blood cells 5 per cent | Control serum unheated (Group O) | Control red blood cells 5 per cent (Group O) | Complement 1:10 (guinea pig) | NaCl 0.85 per cent | Kept at                      | Hemolysis |
|------|--------------------------|--------------------------------------|----------------------------------|--|------------------------------|--------------------|------------------------------|-----------|
| 1    | 0.25                     | 0.1                                  |                                  |  | 0.1                          | 0.05               | 0°C 5 minutes, 37°C 2 hours  | +++       |
| 2    | 0.25                     | 0.1                                  |                                  |  | 0.1                          | 0.05               | 0°C 7 minutes, 37°C 2 hours  | +++       |
| 3    | 0.25                     | 0.1                                  |                                  |  | 0.1                          | 0.05               | 0°C 10 minutes, 37°C 2 hours | +++       |
| 4    | 0.25                     | 0.1                                  |                                  |  | 0.1                          | 0.05               | 0°C 15 minutes, 37°C 2 hours | ++        |
| 5    | 0.25                     | 0.1                                  |                                  |  | 0.1                          | 0.05               | 0°C 20 minutes, 37°C 2 hours | ++        |
| 6    | 0.25                     | 0.1                                  |                                  |  | 0.1                          | 0.05               | 0°C 30 minutes, 37°C 2 hours | +(+)      |
| 7    |                          | 0.1                                  | 0.25                             |  | 0.1                          | 0.05               | 0°C 10 minutes, 37°C 2 hours | 0         |
| 8    |                          |                                      | 0.25                             | 0.1  | 0.1                          | 0.05               | 0°C 10 minutes, 37°C 2 hours | 0         |
| 9    |                          |                                      |                                  | 0.1  | 0.1                          | 0.3                | 0°C 10 minutes, 37°C 2 hours | 0         |
| 10   |                          | 0.1                                  |                                  |  | 0.1                          | 0.3                | 0°C 10 minutes, 37°C 2 hours | 0         |
| 11   | 0.25                     |                                      |                                  | 0.1  | 0.1                          | 0.05               | 0°C 30 minutes, 37°C 2 hours | ++(+)     |
| 12   | 0.25                     | 0.1                                  |                                  |  | 0.1                          | 0.05               | Not chilled, 37°C 2 hours    | 0         |
| 13   | 0.25                     |                                      |                                  | 0.1  | 0.1                          | 0.05               | Not chilled, 37°C 2 hours    | 0         |

Another question upon which discordant results have been reported (6, 7) is the possible effect of CO<sub>2</sub> either as a substitute for chilling or as an auxiliary factor in bringing about union of the hemolysin and the red cells The experiments I have done with CO<sub>2</sub> were entirely negative (8) No effect upon union nor upon hemolysis was observed

That there is probably some undefined factor in the mechanism of the disease appears to be indicated by observations on two of our

# PROTOCOL 4

December 5, 1921 Patient L. O. Blood drawn from arm vein one portion allowed to clot at room temperature serum separated as soon as coagulation was complete and the experiment set up immediately, a second portion was citrated and used for the preparation of the red cell suspension. Control serum and red cell suspension obtained in the same way from a normal adult of the same blood group.

2.0 cc. of the patient's serum was mixed with 2.0 cc. of washed and packed red blood cells of the patient and immersed in melting ice for 15 minutes. The mixture was then centrifuged cold and the supernatant serum quickly pipetted off. This is "Patient's serum 'auto'-absorbed."

The same absorption of the patient's serum in the cold was carried out with an equal volume of washed and packed red blood cells from a normal individual of the same blood group. This is "Patient's serum 'iso'-absorbed."

| Tube | Patient's serum 'auto'-absorbed untreated | Patient's serum 'iso'-absorbed untreated | Patient's serum untreated | Patient's red blood cells 5 per cent | Control red blood cells 5 per cent (Group A) | Control serum untreated (Group A) | Complement 1:10 (guinea pig) | NaCl 0.85 per cent | Kept at                        | Hemolysis |
|------|---|--|---------------------------|--------------------------------------|--|-----------------------------------|------------------------------|--------------------|--------------------------------|-----------|
| 1    | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | 0°C 10 minutes, 37°C. 2 hours  | 0         |
| 2    | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | 0°C 10 minutes, 37°C. 2 hours  | 0         |
| 3    | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | 0°C. 10 minutes, 37 C. 2 hours | 0         |
| 4    | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | 0 C. 10 minutes, 37°C 2 hours  | 0         |
| 5    |   | 0 25                                     |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | 0°C. 10 minutes, 37°C. 2 hours | ++++      |
| 6    |   | 0 25                                     |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | 0°C. 10 minutes 37°C 2 hours   | ++++      |
| 7    |   |  |                           | 0 1                                  |  |                                   | 0 10 3                       |                    | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 8    |   |  |                           | 0 1                                  |  |                                   | 0 10 3                       |                    | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 9    |   |  |                           | 0 1                                  | 0 25   |                                   | 0 10 05                      |                    | 0°C 10 minutes, 37°C. 2 hours  | 0         |
| 10   |   |  |                           | 0 1                                  | 0 25   |                                   | 0 10 05                      |                    | 0°C. 10 minutes, 37 C. 2 hours | 0         |
| 11   | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | Not chilled, 37 C. 2 hours     | 0         |
| 12   | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | Not chilled, 37°C 2 hours      | 0         |
| 13   | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | Not chilled, 37°C. 2 hours     | 0         |
| 14   | 0 25                                      |  |                           | 0 1                                  |  |                                   | 0 10 05                      |                    | Not chilled, 37°C. 2 hours     | 0         |

Titration of the Wasserman reaction on the "auto"-absorbed and the untreated serum used in the above experiment gave the following results

|   | 0.02 cc. | 0.01 cc. | 0.006 cc. | 0.003 cc. | 0.001 cc. | 0.0005 cc. |
|---|----------|----------|-----------|-----------|-----------|------------|
| <i>Untreated serum</i>                        |          |          |           |           |           |            |
| Alcoholic antigen                             | ++++     | ++++     | ++++      | ++++      | +         | 0          |
| Cholesterol antigen                           | ++++     | ++++     | ++++      | ++++      | +         | 0          |
| <i>"Auto"-absorbed serum, not inactivated</i> |          |          |           |           |           |            |
| Alcoholic antigen                             | ++++     | ++++     | ++++      | ++++      | +         | 0          |
| Cholesterol antigen                           | ++++     | ++++     | ++++      | ++++      | ++        | ±          |
| <i>"Auto"-absorbed serum, inactivated</i>     |          |          |           |           |           |            |
| Alcoholic antigen                             | ++++     | ++++     | ++++      | ++++      | ++        | 0          |
| Cholesterol antigen                           | ++++     | ++++     | ++++      | ++++      | +         | 0          |

patients One was a boy of 7 and the other a boy of 8 One had an extreme susceptibility to spontaneous attacks and a low titer of hemolysin in his serum and plasma, the other had paroxysms only after relatively long exposure to low temperatures and a high titer of hemolysin in his serum and plasma There seems to be no explanation for such an inverse relationship between the titer of hemolysin and the susceptibility to attacks unless one postulates some factor as yet undemonstrated The fact that the boy with a low titer of hemolysin and high susceptibility to attacks was subject to very marked vasomotor disturbances while the other boy had none that were apparent,

#### PROTOCOL 5

November 11, 1920 Patient J B Serums, red cell suspensions and complement prepared in the usual way One portion of the patient's serum was inactivated at 55°C for 30 minutes

| Tube | Patient's serum un heated | Patient's serum in activated | Patient's red blood cells 5 per cent | Complement 1 10 (guinea pig) | NaCl 0 85 per cent | Kept at  | Hemolysis |
|------|---------------------------|------------------------------|--------------------------------------|------------------------------|--------------------|--|-----------|
| 1    | cc                        | cc                           | cc                                   | cc                           | cc                 | 0°C 30 minutes, 37°C 2 hours   | +++       |
| 2    | 0 25                      | 0 25                         | 0 1                                  | 0 1                          | 0 05               | 0°C 30 minutes, 37°C 2 hours   | 0         |
| 3    |                           | 0 25                         | 0 1                                  | 0 1                          | 0 05               | 0°C 30 minutes, 37°C 2 hours   | ++        |
| 4    |                           | 0 25                         | 0 1                                  |                              | 0 05               | 0°C 30 minutes Tube warmed to 37°C then 0 1 cc complement added Kept at 37°C 2 hours | +         |

All the usual controls were negative

would suggest that a vasomotor mechanism may have something to do with the hemolysis

From the etiological and from the therapeutic point of view the most important aspect of this disease is its relation to syphilis Many of the reported cases have been in congenital syphilitics The Wasserman reaction is positive in nearly every case and in many of the adult cases late manifestations of acquired syphilis have been present (9, 10) It was of interest therefore to attempt the separation of the auto-hemolysin from the Wassermann reacting substance This has

# PROTOCOL 6

January 27, 1925 Patient G C Blood drawn from arm vein, divided into two portions serum and red cell suspension prepared in the usual way Test set up immediately

| Tube | Patient's serum un-heated | Patient's red blood cells 5 per cent | Complement 1:10 (guinea pig) | NaCl 0.85 per cent | Kept at                        | Hemolysis |
|------|---------------------------|--------------------------------------|------------------------------|--------------------|--------------------------------|-----------|
|      | cc                        | cc                                   | cc                           | cc                 |                                |           |
| 1    | 0.3                       | 0.1                                  | 0.1                          |                    | 0°C. 10 minutes, 37°C. 2 hours | ++++      |
| 2    | 0.25                      | 0.1                                  | 0.1                          | 0.05               | 0°C. 10 minutes, 37°C. 2 hours | ++++      |
| 3    | 0.2                       | 0.1                                  | 0.1                          | 0.1                | 0°C. 10 minutes, 37°C. 2 hours | ++++      |
| 4    | 0.15                      | 0.1                                  | 0.1                          | 0.15               | 0°C. 10 minutes, 37°C. 2 hours | +++       |
| 5    | 0.10                      | 0.1                                  | 0.1                          | 0.2                | 0°C. 10 minutes, 37°C. 2 hours | +++       |
| 6    | 0.05                      | 0.1                                  | 0.1                          | 0.25               | 0°C. 10 minutes, 37°C. 2 hours | +         |
| 7    | 0.01                      | 0.1                                  | 0.1                          | 0.29               | 0°C. 10 minutes, 37°C. 2 hours | ±         |

The usual controls were negative

## *Titration of the Wassermann reaction on the same serum*

|                     | 0.1 cc. | 0.02 cc. | 0.01 cc. | 0.006 cc. | 0.003 cc. | 0.001 cc. |
|---------------------|---------|----------|----------|-----------|-----------|-----------|
| Alcoholic antigen   | ++++    | ++++     | ++++     | ++++      | ++++      | +++       |
| Cholesterol antigen | ++++    | ++++     | ++++     | ++++      | +++       | ++        |

The serum was then left in the ice box for 3 days. Repetition of the above titrations January 30 1925

| Tube | Patient's serum un-heated | Patient's red blood cells 5 per cent | Complement 1:10 (guinea pig) | NaCl 0.85 per cent | Kept at                        | Hemolysis |
|------|---------------------------|--------------------------------------|------------------------------|--------------------|--------------------------------|-----------|
|      | cc.                       | cc                                   | cc.                          | cc.                |                                |           |
| 1    | 0.3                       | 0.1                                  | 0.1                          |                    | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 2    | 0.25                      | 0.1                                  | 0.1                          | 0.05               | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 3    | 0.2                       | 0.1                                  | 0.1                          | 0.1                | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 4    | 0.15                      | 0.1                                  | 0.1                          | 0.15               | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 5    | 0.10                      | 0.1                                  | 0.1                          | 0.2                | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 6    | 0.05                      | 0.1                                  | 0.1                          | 0.25               | 0°C. 10 minutes, 37°C. 2 hours | 0         |
| 7    | 0.01                      | 0.1                                  | 0.1                          | 0.29               | 0°C. 10 minutes, 37°C. 2 hours | 0         |

The usual controls were negative

## *Titration of the Wassermann reaction on the same serum*

|                     | 0.1 cc. | 0.02 cc. | 0.01 cc. | 0.006 cc. | 0.003 cc. | 0.001 cc. |
|---------------------|---------|----------|----------|-----------|-----------|-----------|
| Alcoholic antigen   | ++++    | ++++     | ++++     | ++++      | +++       | ++        |
| Cholesterol antigen | ++++    | ++++     | ++++     | ++++      | +++       | +         |

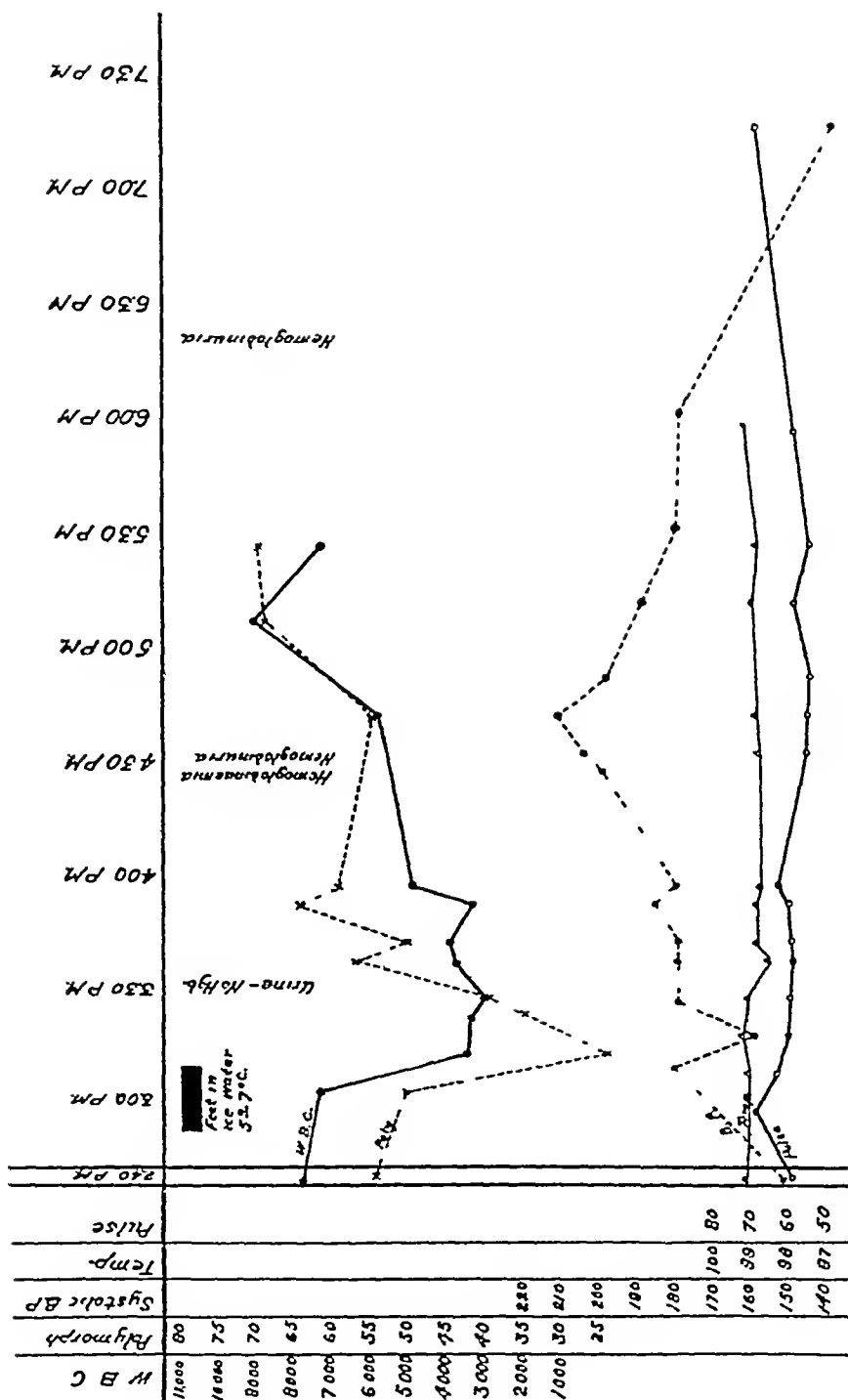


CHART 1 PATIENT J R (APRIL 27, 1922) ARTIFICIALLY INDUCED ATTACK OF HEMOGLOBINURIA

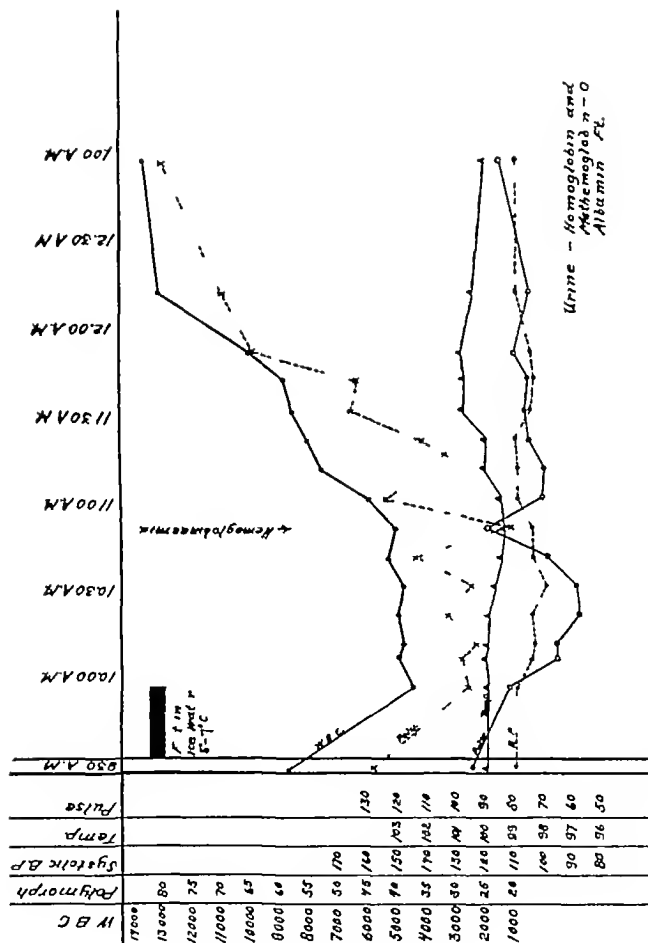


CHART 2 PATIENT J B (JULY 11, 1923) ARTIFICIALLY INDUCED HEMOGLOBINEMIA AND ALBUMINURIA. NO HEMOGLOBINURIA. EXAMPLE OF A LARVAL ATTACK

been done in two ways The auto-hemolysin was completely absorbed out of hemoglobinuric serum and the Wassermann reaction titrated before and after the absorption of the hemolysin (protocol, 4) Such an experiment shows quite regularly that the auto-hemolysin may be

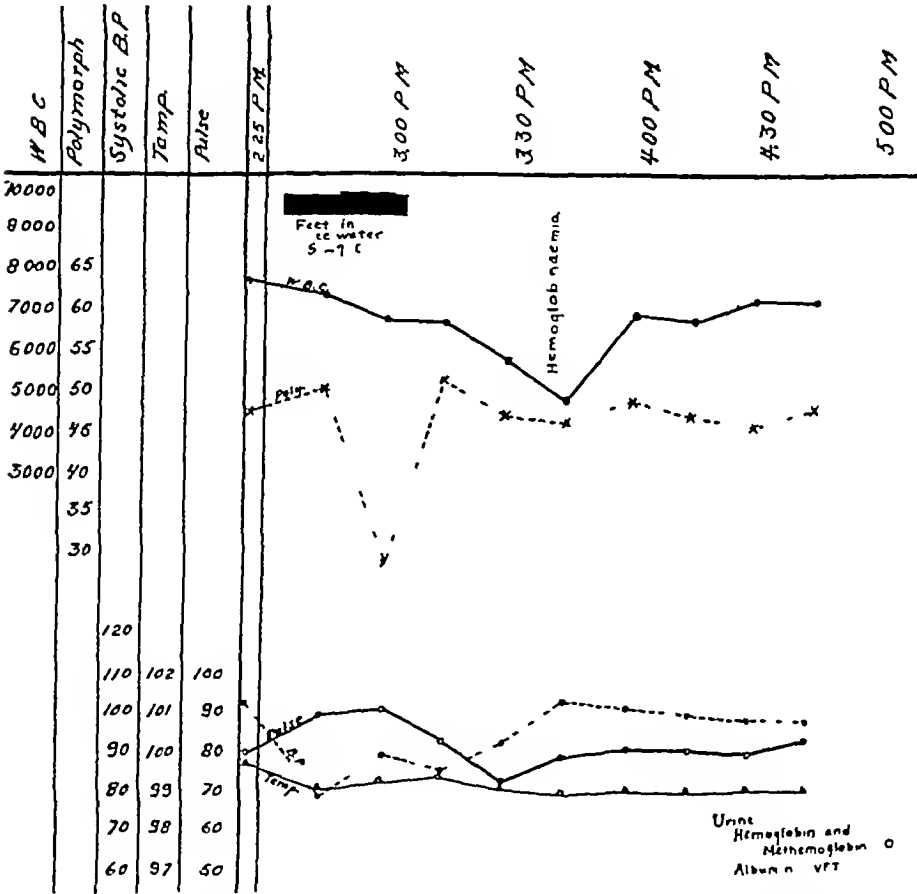


CHART 3 PATIENT G C (DECEMBER 20, 1926) ARTIFICIALLY INDUCED HEMOGLOBINEMIA AND ALBUMINURIA NO HEMOGLOBINURIA EXAMPLE OF A LARVAL ATTACK

completely removed without perceptibly weakening the Wassermann reaction The same result is obtained if hemoglobinuric serum is allowed to stand in the ice-box until the auto-hemolysin has disappeared (protocol 6) It will be found that the Wassermann reacting

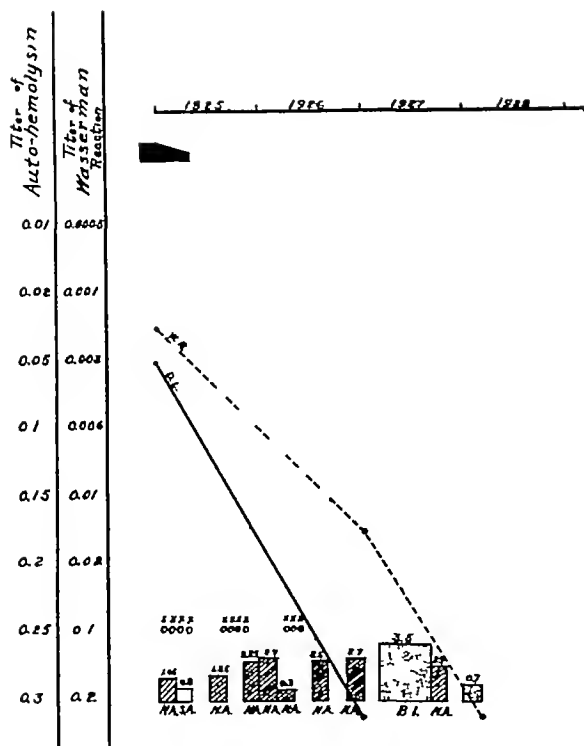


CHART 4 EFFECT OF ANTISYPHILITIC TREATMENT ON AUTO-HEMOLYSIN, W R. AND SYMPTOMS PATIENT G C

The period during which spontaneous attacks of hemoglobinuria occurred is shown in black at the top of the chart. At the bottom of the chart the kind and amount of treatment is indicated A = arsphenamine NA = neoarsphenamine SA = sulpharsphenamine BI = intramuscular and intravenous injections of bismuth The amount of each of these drugs given is shown in grams at the top of the columns representing the different drugs XXX = injections of mercury or intramuscular injections OOO = potassium iodide. Line WR = Wassermann reaction Line DL = Donath Landsteiner reaction

substance shows no significant change. Furthermore, comparison of the titers of the two reactions in a series of patients shows that the titers do not parallel each other. Such results were perhaps to be

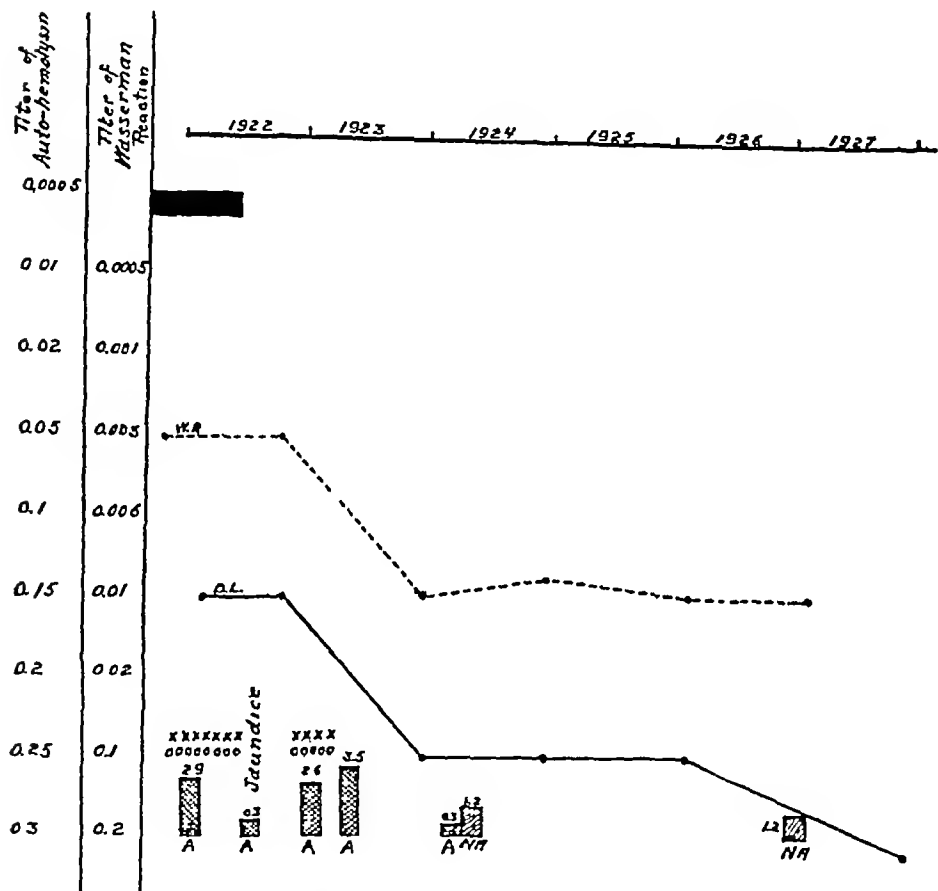


CHART 5 EFFECT OF ANTISYPHILITIC TREATMENT UPON AUTO-HEMOLYSIS, W R AND SYMPTOMS PATIENT L O  
Symbols as in Chart 4

expected from the dissimilarity of the effects of temperature upon the two substances <sup>2</sup>

<sup>2</sup> Burmeister reported experiments in which he found that absorption of paroxysmal hemoglobinuria serum by erythrocytes removed both the auto hemolysin and the Wassermann reacting substance. In view of the opposite results of Moro and Noda (12), Matsuo (10) and Kaznelson (13) and those reported in this paper, Burmeister's conclusions should not be accepted without confirmation.

Certain very striking vasomotor phenomena are often observed during paroxysms of this disease (14) Two of the five patients had Raynaud's syndrome during attacks, one of these was found to have a systolic blood pressure of 250 mm Hg during a spontaneous paroxysm His usual systolic pressure was 140 to 150 mm Hg

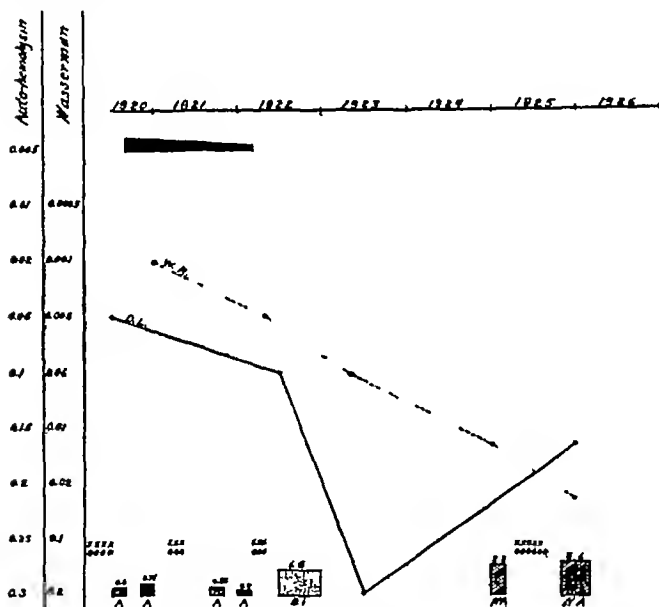


CHART 6 EFFECT OF ANTISYPHILITIC TREATMENT UPON AUTO-HEMOLYSIN, W R AND SYMPTOMS PATIENT J B  
Symbols as in Chart 4

When a paroxysm of hemoglobinuria is artificially induced (Rosenbach test) by immersion of the hands or feet in ice water there may be, in addition to hemoglobinemia and hemoglobinuria, changes in blood pressure, temperature and the leucocyte count, the crise hemoclasique of Widal Chart 1 shows the results of such an experiment The

TABLE 1

Summary of clinical, serological and therapeutic observations on 5 patients with paroxysmal hemoglobinuria

| Case                         | Duration | Seasonal occurrence          | History relating attacks to chilling | Wassermann reaction | Clinical evidence of syphilis                                   | Vasomotor phenomena       | Susceptibility to attacks | Donath and Landsteiner |       | Kosenbach test | Hemoclastic reaction | Kind of treatment          | Result of treatment  | Blood group | Remarks  |
|------------------------------|----------|------------------------------|--------------------------------------|---------------------|---|---------------------------|---------------------------|------------------------|-------|----------------|----------------------|----------------------------|--|-------------|--|
|                              |          |                              |                                      |                     |   |                           |                           | Result                 | Titre |                |                      |                            |  |             |  |
| J. H. (F)<br>Age 9           | 4½ years | Fall, winter, spring         | +                                    | +++++               | Hutchinson teeth, enlarged liver                                | None                      | ++                        | +                      | 0.05  | #              | +                    | Anti luetic                | Cessation of attacks. Donath and Landsteiner and Wassermann reaction still positive                      | A           |  |
| I. O. (M)<br>Age 58          | 1 year   | Winter                       | +                                    | ++-+                | Enlarged tender and irregular liver                             | None                      | ++                        | +                      | 0.15  | +              | #                    | Irregular anti luetic      | Cessation of attacks. Donath and Landsteiner and Wassermann reaction still positive                      | A           | Donath and Landsteiner later negative Wassermann reaction still positive |
| J. R. (M)<br>Negro<br>Age 40 | 1½ years | Fall, winter, spring         | +                                    | +++++               | None  | Raynaud's syndrome        | +++                       | +                      | 0.07  | +              | +                    | Very irregular anti luetic | No effect  | O           | Died lobar pneumonia. Type III 1926                                      |
| C. C. (M)<br>Negro<br>Age 8  | 6 weeks  | Winter                       | Indefinite                           | +++++               | Hutchinson teeth?   | None                      | +++                       | +                      | 0.003 | #              | +                    | Intensive anti luetic      | Prompt cessation of attacks. Later Donath and Landsteiner and Wassermann reaction negative               | AB          |  |
| F. V. (M)<br>Age 7           | 4 years  | All the year, more in winter | +                                    | +++++               | Saddle nose<br>Hutchinson teeth, large liver and spleen, anemia | Hives, Raynaud's syndrome | +++                       | +                      | 0.2   | Not done       | Not done             | Irregular anti luetic      | General condition improved. At attacks continued Donath and Landsteiner and Wassermann reaction positive | O           |  |

total white count and the percentage of polymorphonuclears show an abrupt drop after the feet have been in ice water for a few minutes. Synchronous with the decrease in polymorphonuclear cells there is an increase in the percentage of lymphocytes. After one to two hours the leucocytes return to normal, and this may be followed by a leucocytosis due to an increase above normal of the polymorphonuclear cells (chart 2). The blood pressure may be unaffected or may show a gradual increase over a period of about two hours following the chilling (chart 1). Charts 2 and 3 are examples of larval attacks artificially induced. In these experiments hemoglobinemia occurred but there was no hemoglobinuria. Nevertheless, the characteristic changes in the blood picture were observed.

The five patients on whom observations have been made were all given anti syphilitic treatment (15). Two of these patients did not return with sufficient regularity to receive adequate treatment. The results of treatment on the other three patients are shown in charts 4, 5, and 6. It is perhaps noteworthy that the patient (G C) receiving the most intensive treatment showed the most prompt cessation of symptoms and the earliest disappearance of the Donath and Landsteiner and the Wassermann reactions, even though the Donath and Landsteiner had a higher titer in this patient than in any other patient of the series.

Table 1 summarizes some of the clinical, serological and therapeutic observations on the group of patients studied.

#### SUMMARY

Paroxysmal hemoglobinuria is a manifestation of late syphilis, it is characterized by the presence of an extremely labile serum auto-hemolysin which is distinct from the Wassermann reacting substance. Union of the hemolysin with the red blood cells occurs only at low temperatures, short chilling is more effective than long periods at low temperatures, the auto hemolysin and the iso-hemolysin are probably identical.  $\text{CO}_2$  has been reported to act as a substitute for chilling but such an effect was not demonstrable in the patients of this series. Artificial production of a paroxysm causes hemoglobinemia with or without hemoglobinuria, frequently a sudden drop in the total white count and the percentage of polymorphonuclear cells with a subsequent

leucocytosis and increase of the percentage of polymorphonuclears, an elevation of blood pressure and a rise in the temperature. Intensive anti-leucetic treatment may be expected to cause cessation of symptoms, disappearance of the auto-hemolysin and conversion of a positive to a negative Wassermann reaction in the order named

#### TECHNIQUE

Our routine method of doing the Donath-Landsteiner reaction has been to draw blood from an arm vein, allow it to clot at room temperature, to separate the serum by centrifugalization as soon as coagulation was complete and to set up the test immediately. In a few instances the serum was kept in the ice box over night and the test set up 18 to 24 hours after drawing the blood. The rapid deterioration of the lysin of some of these patients even at ice box temperature, however, makes it advisable to set up the test as soon as the serum has been separated. A 5 per cent suspension of red blood cells was used unless there was a special reason for a heavier or lighter suspension. The suspension was prepared by washing citrated blood three times with a large volume of 0.85 per cent NaCl and suspending the packed cells in 0.85 per cent NaCl. Owing to the thermolability of some of these lysins the serum was used without heating, but to insure the presence of adequate complement fresh pooled guinea pig serum diluted 1:10 was added. The volume was made up to 0.5 cc with 0.85 per cent NaCl. The chilling was accomplished by placing the tubes in finely crushed ice. The usual time for chilling has been 10 minutes. The warming of the tubes was done by placing them in a water bath at 37°C.

Six protocols illustrating the technical procedures followed in studying some of the questions referred to in the text are included. These are representative of a large number of similar experiments performed during the period of nine years that the patients of this series have been studied.

#### BIBLIOGRAPHY

- 1 Donath, J, and Landsteiner, K, *Munch med Wchnschr*, 1904, li, 1590  
Ueber paroxysmalen Hämoglobinurie
- 2 Rosenbach, O, *Berl klin Wchnschr*, 1880, xvii, 132 and 151. Beitrag zur Lehre von der periodischen Hämoglobinurie
- 3 Yorke, W, and Macfie, J W S, *Brit J Exp Path*, 1921, ii, 115. The Mechanism of Autolysis in Paroxysmal Hemoglobinuria
- 4 Moss, W L, *Johns Hopkins Hospital Bull*, 1911, xxi, 238. Paroxysmal Hemoglobinuria, Blood Studies in Three Cases
- 5 Widal, F, Abrami, P, and Brissaud, E, *Sem Med*, 1913, xxxiii, 585. Recherches sur l'hémoglobinurie paroxystique "a frigore"
- 6 Hijmans van der Bergh, A A and Hijmans, C, *Berl klin Wchnschr*, 1909, 1251 and 1609. Untersuchung über die Hamolyse bei der paroxysmalen Hämoglobinurie

- 7 Donath, J , and Landsteiner, K., *Ergebn d Hygiene, Bakt etc.*, 1925, vii, 184 Über Kälthämoglobinurie
- 8 Mackenzie G M , *Proc Soc Exp Biol and Med* , 1925, xxi, 278 Note on the Mechanism of Paroxysmal Hemoglobinuria
- 9 Meyer, E , and Emmerich, E , *Deutsche Arch f Klin Med* , 1909, xcv, 287 Über paroxysmale Hämoglobinurie
- 10 Matsuo, J , *Deutsche Arch f Klin Med* , 1912, cvii, 335 Über die klinischen und serologischen Untersuchungen der paroxysmalen Hämoglobinurie, zugleich ein Beitrag zur Kenntnis der Isolyse
- 11 Burmeister, J , *Ztschr f Klin. Med* , 1921, xci, 19 Ueber paroxysmale Hämoglobinurie und syphilis, zugleich ein Beitrag zur Problem der Erhaltungskrankheiten.
- 12 Moro, E , and Noda, S , *Münch med. Wchnschr* , 1909, lvi, 545 Paroxysmale Hämoglobinurie und Hämolyse in Vitro
- 13 Kaznelson, P , *Deutsche Arch f klin Med.*, 1921, cxxviii, 46 Beobachtungen über paroxysmale Kältehemoglobinurie und Kälteikterus
- 14 Browning, C H., and Watson, H F , *Internat Clinics* 1913, ii, 111 Paroxysmal Hemoglobinuria Its Relation to Syphilis in the light of the Wassermann reaction
- 15 Kumagai, T , and Namba, M *Deutsche Arch f Klin med* , 1927, clvi, 257 Weitere Beiträge zur Kenntnis der paroxysmalen Hämoglobinurie



# A QUANTITATIVE METHOD FOR THE ESTIMATION OF PEPSIN

BY W SCOTT POLLAND AND ARTHUR L. BLOOMFIELD

*(From the Department of Medicine Stanford University Medical School, San Francisco)*

*(Received for publication October 8, 1928)*

The quantitative estimation of ferments is beset by many difficulties which do not obtain in the case of substances which can be directly weighed or measured. Since, in the present state of our knowledge, ferments can only be recognized through their effects, and since these effects vary greatly with many conditions, it has been difficult to develop standard methods which possess reasonable accuracy and at the same time are not too cumbersome.

The effects of ferment action vary with the time over which the reaction proceeds, the concentration of the substrate and the concentration of the enzyme as well as with other factors. Most of the methods have been based on the principle of varying one factor while others are kept constant, and the strength of the enzyme is usually expressed in terms of the degree to which the substrate has been effected. The details of the many procedures which have been devised may be read in Oppenheimer's elaborate treatise (1) or in the briefer compendium of Waksman and Davison (2). With regard to pepsin the usual procedure has been to allow decreasing quantities of ferment (gastric juice) to act on a uniform amount of substrate (such as a soluble protein) over a standard length of time and to determine the smallest amount of ferment which completely digests (as digestion may be defined) the unit of substrate. Thus, if 0.01 cc of ferment completely digests the unit of substrate it is assumed that 1 cc of ferment has the power of digesting 100 units. As various workers have shown, however, this assumption is entirely erroneous (3) since activity does not vary in direct proportion to the concentration of ferment. Methods based on this general plan must therefore be discarded. Northrup and Hussey recently have pointed out (4) that the time required to cause a given percentage change in the viscosity

of a solution of substrate is nearly inversely proportional to the amount of enzyme present. On this basis they have developed a practical method for the measurement of peptic or tryptic activity. Special precautions are necessary, however, in the preparation of the substrate.

The present method, which was devised during the course of studies of peptic activity of gastric juice subsequently to be reported, is in accord with the established principles of ferment action and in practice has been found to be reasonably simple and accurate. In brief, increasing dilutions of ferment (gastric juice) are allowed to act on a uniform amount of substrate (soluble protein) for a uniform length of time (one-half hour at  $37^{\circ}\text{C}$ ). Instead of determining the greatest dilution which gives complete digestion the percentages of substrate digested by various dilutions of ferment are estimated. From these figures a curve can be constructed which shows at its peak the maximum degree of ferment activity under the conditions of the experiment.

#### PROCEDURE

A standard solution of protein (substrate) is prepared as follows: 2 grams of edestin (Special Chemicals Company, Waukegan, Ill.) are placed in a small beaker. To this is added, without stirring, 50 cc of 0.3 N hydrochloric acid. The mixture is placed in a water bath ( $100^{\circ}\text{C}$ ) for one-half hour. Occasionally a minute residue of undissolved edestin remains. This is filtered off with gauze and dissolved in 10 cc of acid which is then added to the edestin already dissolved. A few drops of thymol blue are next introduced as indicator and 20 cc N/10 barium hydroxide are added. The mixture is then placed in a 100 cc volumetric flask which is filled to the 100 cc mark by adding N/100 HCl. The reaction is tested colorimetrically and adjusted so that the final mixture is a 2 per cent solution of edestin with a pH of approximately 2.0. This mixture may be kept on ice apparently unaltered (see below) for at least several weeks. It should be well shaken before being used.

In a series of small test tubes increasing dilutions of ferment (gastric juice) in N/100 HCl are set up. The ratio of successive dilutions may be as 1, 2, 4, etc., or otherwise as desired. In any case the total

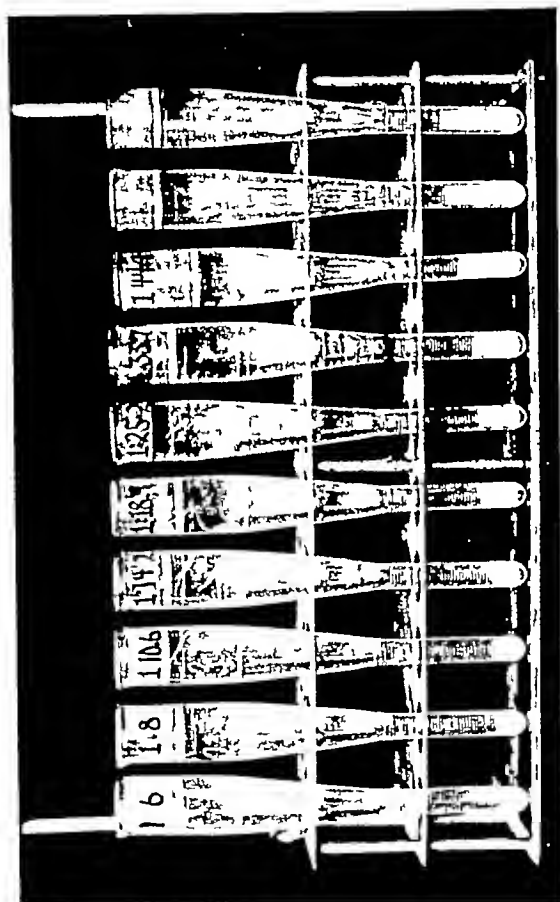


FIG 1 PHOTOGRAPH OF RACK OF TUBES FROM TEST SHOWN IN CHART 5, A

volume of diluted ferment in each tube is 1.0 cc. Five tubes containing 1.0 cc. of  $N/100$  HCl but no ferment are added to the series as controls. To each tube 1 cc. of the edestin solution is next added and the tubes are then shaken and placed in the water bath at  $37^{\circ}\text{C}$  for exactly one-half hour. Throughout the procedure accurately calibrated volumetric pipettes are used and the digestion is carried out at approximately pH 2.0. After incubation 1 cc. of 15 per cent trichloroacetic acid is added to each tube. Unless complete digestion has taken place the remaining unsplit protein is precipitated. The contents of each tube are next transferred to a specially graduated centrifuge tube and the volume is made up to a total of 6.5 cc. The tubes have a capillary stem accurately graduated to 0.01 cc.<sup>1</sup> The tubes are next allowed to stand for several hours or preferably overnight at room temperature to allow swelling of the precipitate to come to an equilibrium and they are then centrifuged at 1600 revolutions for exactly 10 minutes. An automatic timing device attached to the centrifuge is very convenient. The bulk of the precipitate in the control tubes and in the test tubes is then read on the scale and the degree of digestion which has taken place with each dilution of ferment can be expressed in percentages in relation to the control. If, for example, the average of the control tubes which contained no ferment shows a precipitate to the 0.30 mark and if the tube containing 1 cc. of ferment shows precipitate to the 0.15 mark and if the control tube represented 300 mgm. of edestin, then 1 cc. of ferment has digested  $\frac{0.15}{0.30} = \frac{1}{2} = 150$  mgm. of edestin. The calculations can best be demonstrated by an illustrative protocol.

*Experiment 1* Increasing dilutions of a solution of commercial pepsin (Squibb's) in  $N/100$  HCl were set up as shown in the protocol (table 1). To each tube 1 cc. of a 2 per cent solution of edestin in  $N/100$  HCl was then added, the tubes were incubated in the water bath at  $37^{\circ}\text{C}$  for 30 minutes, trichloroacetic acid was added and the tubes were centrifuged. The second column (readings of protein precipitated) shows the reading of the height of the undigested precipitated protein in the capillary stem of the graduated centrifuge tube. The figures in the third and fourth columns are self-evident.

<sup>1</sup> This tube devised by Dr. Thomas Addis for measuring small amounts of precipitate is made by the A. H. Thomas Company. (See fig. 1.)

TABLE 1  
Data from a solution of commercial pepsin (Squibb)

|  | Dilutions of pepsin in N/100 HCl |       |        |       |        |        |        |        |        |  |  |
|--|----------------------------------|-------|--------|-------|--------|--------|--------|--------|--------|--|--|
|  | 1 6                              | 1 8   | 1 1066 | 1 142 | 1 18.9 | 1 25.2 | 1:33 7 | 1 44.9 | 1 59.8 |  |  |
| Readings of precipitated protein on graduated scale of centrifuge tube   | 0 060                            | 0 071 | 0 080  | 0 094 | 0 104  | 0 131  | 0 174  | 0 211  | 0 243  |  |  |
| Amounts of protein digested (difference between control and specimen reading)  | 0 190                            | 0 179 | 0 170  | 0 156 | 0 146  | 0 119  | 0 076  | 0 039  |        |  |  |
| Percentage of protein digested $\left( \frac{\text{Amount digested}}{\text{Control}} \right)$  | 76 0                             | 71 6  | 68 0   | 62 4  | 58 4   | 47 6   | 30 4   | 15 6   |        |  |  |
| Mgm. edestin digested by 1 cc. of 1 per cent pepsin solution under varying conditions (concentration edestin X per cent digested X dilution of specimen) | 91 2                             | 114 6 | 145 0  | 177 3 | 221 3  | 240 3  | 205 0  | 140 0  |        |  |  |

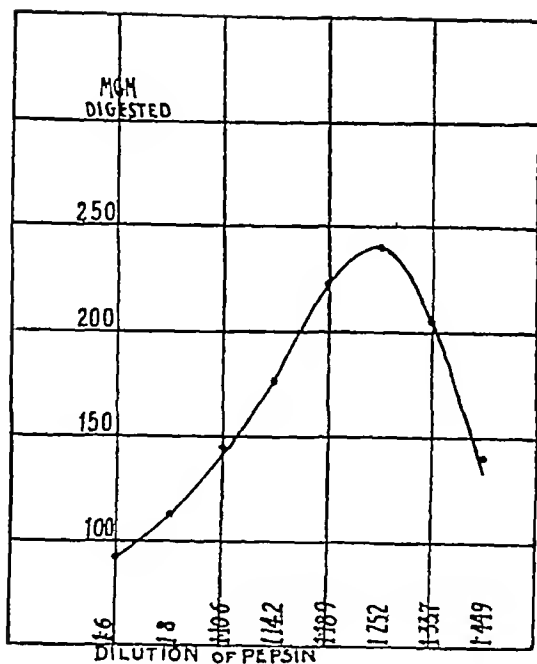


CHART 1 CURVE SHOWING AMOUNTS OF EDESTIN (MILLIGRAMS) DIGESTED BY 1 CC OF 1 PER CENT PEPsin SOLUTION UNDER VARIOUS CONDITIONS OF DILUTION

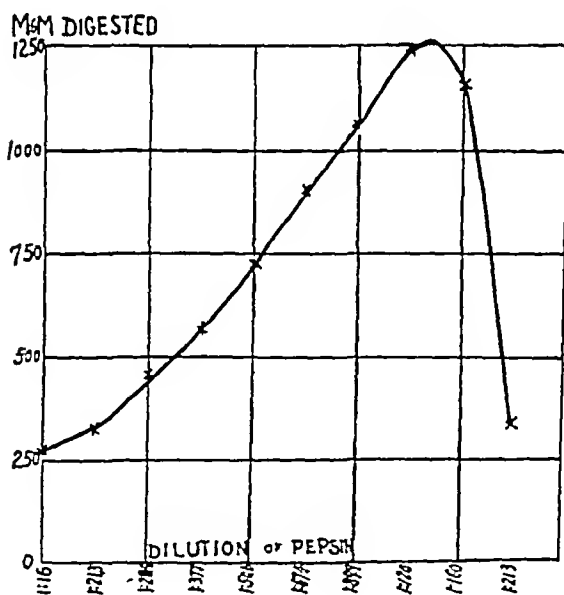


CHART 2 CURVE SHOWING AMOUNTS OF EDESTIN (MILLIGRAMS) DIGESTED BY 1 CC OF GASTRIC JUICE UNDER VARIOUS CONDITIONS OF DILUTION

TABLE 2  
Data from human gastric juice obtained after histamine stimulation

|  | Dilutions of pepsin in N/100 HCl |        |        |        |        |        |        |        |        |       |
|--|----------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
|  | 1 16                             | 1 21.3 | 1 28.4 | 1 37.7 | 1 50.6 | 1 67.4 | 1 89.9 | 1 120  | 1 160  | 1 213 |
| Readings of precipitated protein on graduated scale of centrifuge tube   | 0 040                            | 0 042  | 0 050  | 0 060  | 0 070  | 0 080  | 0 105  | 0 120  | 0 160  | 0 230 |
| Amounts of protein digested (difference between control and specimen reading)  | 0 210                            | 0 208  | 0 200  | 0 190  | 0 180  | 0 170  | 0 155  | 0 130  | 0 090  | 0 020 |
| Percentage of protein digested ( $\frac{\text{Amount digested}}{\text{Control}}$ )   | 84 0                             | 83 2   | 80 0   | 76 0   | 72 0   | 68 0   | 62 0   | 52 0   | 36 0   | 8 0   |
| Mgmn edestin digested by 1 cc. of 1 per cent pepsin solution under varying conditions (concentration edestin X per cent digested X dilution of specimen) | 268 8                            | 354 2  | 454 9  | 573 0  | 728 4  | 917 0  | 1114 0 | 1248 0 | 1152 0 | 340 9 |

The last column gives the actual number of milligrams of edestin digested in each tube multiplied by the dilution of pepsin so that the final results are all expressed in terms of digestive power of 1 cc of undiluted 1 per cent pepsin solution. They are shown graphically in chart 1 by plotting the total amount of edestin digested by 1 cc of

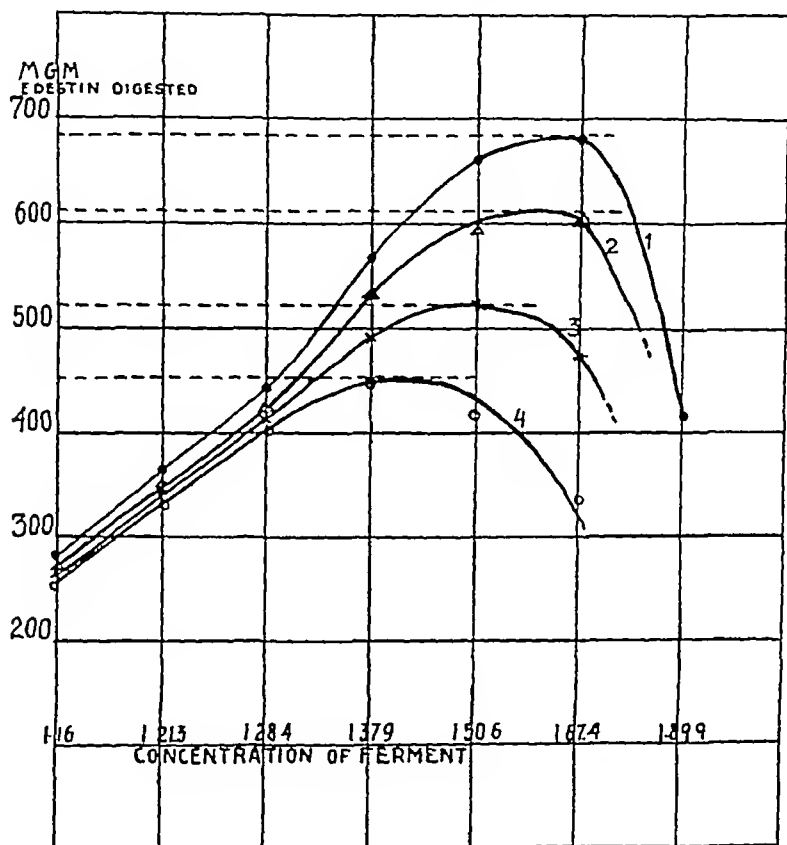


CHART 3 CURVES SHOWING TITRATION OF PEPSIN IN GASTRIC JUICE OBTAINED BEFORE AND AFTER HISTAMINE STIMULATION

pepsin in various dilutions against the dilution. It is evident that as the dilution of pepsin is increased relatively more digestion takes place until a certain optimum point (the peak of the curve) is reached. Beyond this peak further dilution of pepsin is followed by an abrupt lessening of digestive power. If one determines by the above method

enough points to construct a curve it is evident that the high point will indicate the optimum digestive power of the specimen under consideration and will allow comparison with other specimens examined in the same way

*Experiment 2* Chart 2 and table 2 show the results of another experiment made with a specimen of gastric juice obtained after histamine stimulation from a normal human stomach. The shape of the curve is similar to that obtained in experiment 1, and indicates that under optimum conditions 1 cc of the gastric juice in question digests about 1250 mgm of edestin. One cubic centimeter of a 1 per cent solution of commercial pepsin, on the other hand (experiment 1), digested only about 242 mgm. of edestin. The activity of the two specimens may therefore be compared, the relation being as 242:1250

In work with normal human gastric juice we have found that in order to construct a satisfactory curve ten tubes are necessary, beginning with a dilution of 1:16 which is increased by  $1/4$  in each successive tube. In anacidity cases in which pepsin is diminished it is necessary to begin with the undiluted juice, but increasing dilutions in the ratio of 1:2 are adequate. Chart 3 shows the results with specimens of pure gastric juice obtained from the same person before and after histamine stimulation. Curve 1 is from a specimen before histamine, curves 2, 3 and 4 are from specimens obtained 30, 40 and 50 minutes after histamine. The optimum digestive power of 1 cc of juice from the various specimens is, in terms of milligrams of edestin, 682, 611, 521 and 452, figures which may be directly compared

#### CONTROL TESTS

1 *Repeated tests with the same substrate to test its stability* A lot of substrate was prepared according to the method outlined above. At weekly intervals it was tested against a solution of pepsin freshly prepared from the commercial powder. Chart 4 shows the results of the three tests which were practically identical. One may conclude that the substrate remains essentially unaltered over a period of at least two weeks.

2 Two lots of substrate were prepared and tested with the same pepsin solution at the same time. The results shown in chart 5

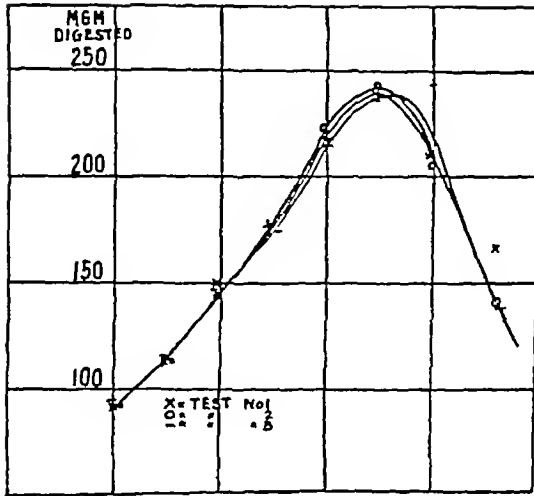


CHART 4 TITRATION OF PEPSIN AGAINST THE SAME LOT OF SUBSTRATE AT WEEKLY INTERVALS

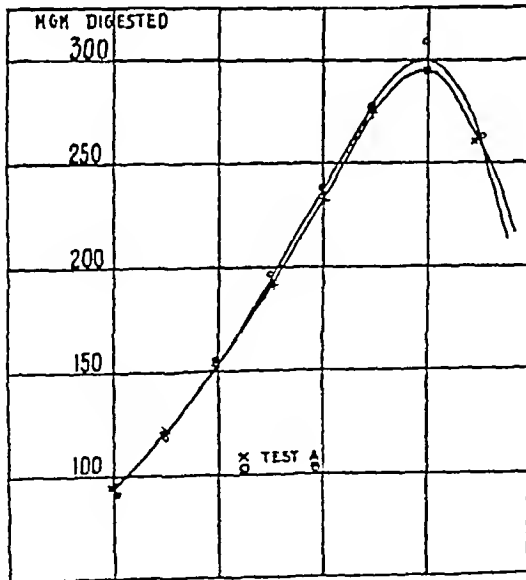


CHART 5 TITRATION OF PEPSIN AGAINST TWO LOTS OF SUBSTRATE

indicate that various lots of substrate are essentially identical and may therefore be used in comparative tests of peptic activity

It is difficult to estimate in mathematical terms the exact degree of accuracy of the method. As is the case with all ferment titrations, unless the most accurate physical chemical methods are employed, a certain amount of error is unavoidable. Our controls indicate that this error is usually not over five per cent.

#### SUMMARY

A method for comparing the peptic activity of specimens of gastric juice which eliminates certain fundamental errors of some of the older methods is described. In the following paper the results of the application of the method in the study of gastric physiology are exposed.

#### BIBLIOGRAPHY

- 1 Oppenheimer, C, *Die Fermente und ihre Wirkungen*. Vol II. Leipsic, 1926
- 2 Waksman, S A, and Davison, W C, *Enzymes, Properties, Distribution, Methods and Applications*. Baltimore, 1926
- 3 Northrup, J H, *The Dynamics of Pepsin and Trypsin*. The Harvey Lectures, 1925-26, p 36
- 4 Northrup, J H, and Hussey, R G, *J Gen Physiol*, 1922-23, v, 353. A Method for the Quantitative Determination of Trypsin and Pepsin



# QUANTITATIVE MEASUREMENTS OF PEPSIN IN GASTRIC JUICE BEFORE AND AFTER HISTAMINE STIMULATION

By W SCOTT POLLAND AND ARTHUR L. BLOOMFIELD

*(From the Department of Medicine, Stanford University Medical School, San Francisco)*

*(Received for publication October 8, 1928)*

While the chloride and other inorganic constituents of the gastric juice have been thoroughly studied by many workers, few observations on pepsin are on record. The inadequacy of knowledge about this substance is doubtless due to lack of suitable methods inasmuch as accurate pepsin determinations, as a rule, have not been available in the clinic. In another paper (1) we have described a quantitative method for the estimation of pepsin which has been found to be serviceable and fairly accurate and the present report deals with the application of this procedure to the study of gastric secretion in patients.

During the course of analyses of pure gastric juice, obtained both with the stomach at rest and after histamine stimulation, it was found (2) that the secretion of various constituents did not run parallel with one another. Concentration of chloride and total output of chloride rose after stimulation but concentration of nitrogen and of fixed base fell although the total output was somewhat increased at the height of secretion. With regard to pepsin, then, we desired to determine (a) the actual amounts put out by normal people and those with gastric disease, and (b) whether there was a quantitative relation between the curve of pepsin secretion and that of other elements such as nitrogen. No data bearing on either of these points were found in the literature since such pepsin estimations as are recorded have usually been made on mixtures of gastric juice and test meal and not on pure gastric juice. The true relations are therefore obscured. Lim, Matheson and Schlapp (3) report some observations on pepsin before and after histamine stimulation by estimating the time necessary to digest an egg white suspension. They state that the concen-

tration is usually little altered after stimulation although it is sometimes greatly increased and that no definite relationship has been found with the other constituents of the gastric juice. This is at variance with our findings.

### PROCEDURE

The subjects were all in bed in the hospital. After an over-night fast, a duodenal tube was inserted into the stomach and the fasting contents were withdrawn. The total secretion over ten-minute periods before and after an injection of histamine (0.1 mgm per 10

TABLE 1  
*Data from Case 1*

| Volume of specimen | Appearance                          | Titratable acidity |       | Nitrogen concentration | Pepsin                                 |   |
|--------------------|-------------------------------------|--------------------|-------|------------------------|--|---|
|                    |                                     | Free               | Total |                        | Mgm edestin digested by 1 cc. of juice | Total mgm digested per 10 minute period |
| cc                 |                                     |                    |       | mgm per 100 cc         |  |   |
| 35                 | Clear—some mucus (fasting contents) |                    |       |                        |  |   |
| 13                 | Clear, limpid, some mucus           | 25                 | 35    | 90.8                   | 735                                    | 9,490                                   |
| Histamine 0.6 mgm  |                                     |                    |       |                        |  |   |
| 10                 | Clear, thin, some mucus             | 85                 | 98    | 106                    | 1,540                                  | 15,300                                  |
| 39                 | Clear, thin, some mucus             | 114                | 120   | 59.8                   | 1,240                                  | 48,750                                  |
| 32                 | Clear, thin, some mucus             | 126                | 132   | 42.2                   | 790                                    | 25,600                                  |
|                    | Part of specimen bile stained       | 128                | 132   |                        | 710                                    |   |
| 24                 | Clear                               | 128                | 132   | 42.2                   | 690                                    | 16,500                                  |

kilos body weight) was then collected exactly as previously described. (2) The following determinations were made: (a) titratable acidity, (b) pepsin, and (c) total nitrogen. Titratable acidity was determined with di-methyl in the usual way, total nitrogen (Kjeldahl) is expressed in milligrams per 100 cc, and pepsin in terms of the number of milligrams of edestin digested by one cubic centimeter of gastric juice in 30 minutes.

### RESULTS

*Case 1.* A man, age 27, was convalescent from a mild acute enteritis. He was to all intents and purposes well and had never had

any symptoms of gastric disease The results of the examination are shown in table 1 and chart 1 The volume of secretion, the acid

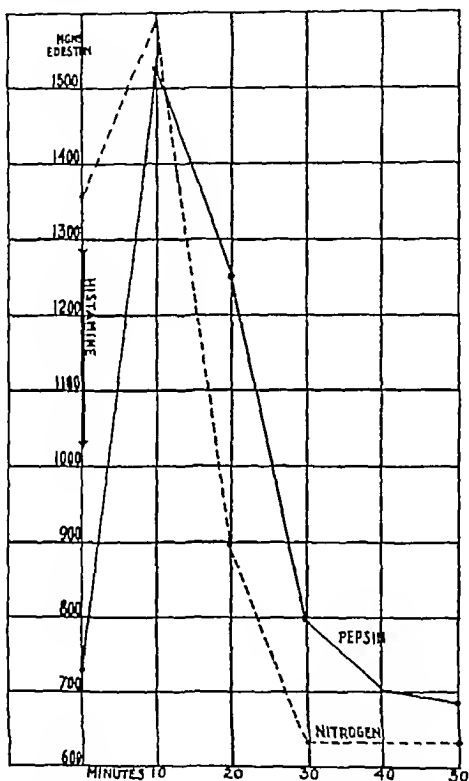


CHART 1 CURVES OF NITROGEN AND PEPSIN CONCENTRATIONS FROM CASE 1 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

values, and the nitrogen values which show the typical fall in concentration after stimulation were all normal The degree of peptic

activity is measured in terms of milligrams of edestin digested by 1 cc of juice. It is seen that the concentration of pepsin not only falls after stimulation in spite of increased volume of secretion, but that the curve of pepsin concentration at various intervals almost parallels that of nitrogen when both are reduced to a corresponding scale by multiplying the nitrogen values (milligrams per 100 cc) by 15. Unless otherwise indicated, nitrogen values are expressed in this way in all the charts.

TABLE 2  
*Data from case 2*

| Volume of specimen | Appearance                  | Titratable acidity |       | Nitrogen concentration | Pepsin                                |   |
|--------------------|-----------------------------|--------------------|-------|------------------------|---------------------------------------|---|
|                    |                             | Free               | Total |                        | Mgm edestin digested by 1 cc of juice | Total mgm digested per 10 minute period |
| cc                 |                             |                    |       | mgm per 100 cc         |                                       |   |
| 20                 | Colorless, mucoid           |                    |       |                        |                                       |   |
| 18                 | Colorless, mucoid           | 45                 | 69    | 102.0                  | 1,730                                 | 31,140                                  |
| Histamine 0.5 mgm  |                             |                    |       |                        |                                       |   |
| 15                 | Thin, colorless, some mucus | 86                 | 107   | 81.0                   | 1,610                                 | 24,150                                  |
| 18                 | Thin, colorless, some mucus | 115                | 128   | 58.5                   | 1,030                                 | 18,540                                  |
| 19                 | Thin, colorless, some mucus | 120                | 131   | 49.5                   | 860                                   | 16,340                                  |
| 15                 | Thin, colorless, some mucus | 126                | 138   | 61.5                   | 977                                   | 14,620                                  |
| 11                 | Thin, colorless, some mucus | 118                | 130   | 63.0                   | 1,010                                 | 11,110                                  |

*Case 2* A man, age 24, East Indian, entered the hospital for nervousness. No evidences of organic disease of any sort were found. The results of the examination are shown in table 2 and chart 2. The volume of secretion, the acidity and the nitrogen output were all normal. As in case 1, the concentration of pepsin fell markedly after stimulation and paralleled the fall in nitrogen concentration very closely.

*Case 3* A man of 23 years, entered the Hospital for minor complaints and showed no evidence of organic disease. The volume of gastric secretion was rather high but not abnormal, acidity and nitrogen were within the usual range. Here again, after stimulation,

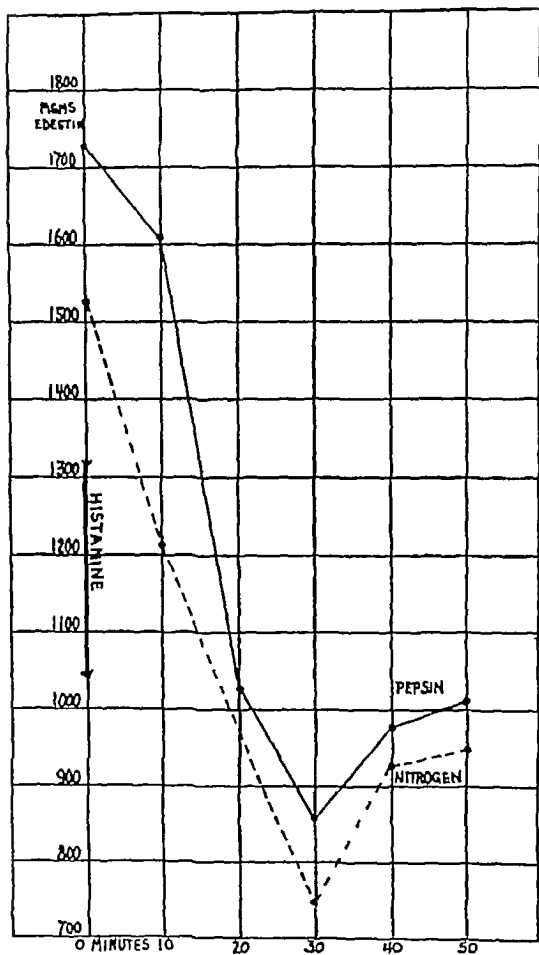


CHART 2 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 2 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

there was a striking fall in concentration of pepsin which ran parallel to the fall in nitrogen (table 3, chart 3)

*Case 4* A man, age 19, had disseminated lupus erythematosus. There were no gastric symptoms nor evidences of disease of the stomach. The volume of secretion, acidity and concentration of nitrogen were within normal limits. In this case again the concentration of pepsin fell after stimulation and paralleled the nitrogen concentration although the curves do not correspond as closely as in

TABLE 3  
*Data from case 3*

| Volume of specimen | Appearance                            | Titratable acidity |       | Nitrogen concentration | Pepsin                                 |   |
|--------------------|---------------------------------------|--------------------|-------|------------------------|--|---|
|                    |                                       | Free               | Total |                        | Mgm edestin digested by 1 cc. of juice | Total mgm digested per 10 minute period |
| cc                 |                                       |                    |       | mgm per 100 cc         |  |   |
| 23                 | Slightly bile tinged fluid some mucus |                    |       |                        |  |   |
| 8                  | Colorless—some mucus                  | 35                 | 52    | 112.5                  | 1,495                                  | 11,960                                  |
| Histamine 0.6 mgm  |                                       |                    |       |                        |  |   |
| 12                 | Colorless—considerable mucus          | 75                 | 90    | 127.5                  | 2,075                                  | 24,900                                  |
| 23                 | Water clear fluid                     | 110                | 116   | 58.5                   | 1,220                                  | 28,060                                  |
| 42                 | Water clear fluid                     | 121                | 126   | 46.5                   | 720                                    | 30,240                                  |
| 32                 | Water clear fluid                     | 118                | 122   | 36.0                   | 605                                    | 19,200                                  |
| 31                 | Water clear fluid                     | 103                | 112   | 46.5                   | 660                                    | 20,460                                  |

the three previous cases. The maximum concentration of pepsin, furthermore, is considerably lower than in cases 1, 2 and 3.

*Case 5* A man of 32 had a penetrating ulcer of the lesser curvature of the stomach. The acidity was within normal limits but the volume of gastric secretion was distinctly high. The pepsin concentration again runs parallel to the nitrogen concentration but is lower than in the normal cases 1, 2 and 3. The low concentration of pepsin is largely explained by the high volume of secretion, the total output of pepsin is not low (see chart 9).

*Case 6* A man, age 32, was convalescent from a mild attack of influenza. There were no digestive symptoms and the temperature

had been normal for several days at the time of the test. The findings on gastric analysis were of interest since the acid values were distinctly low, while the volume of secretion was well above the average. Fur-

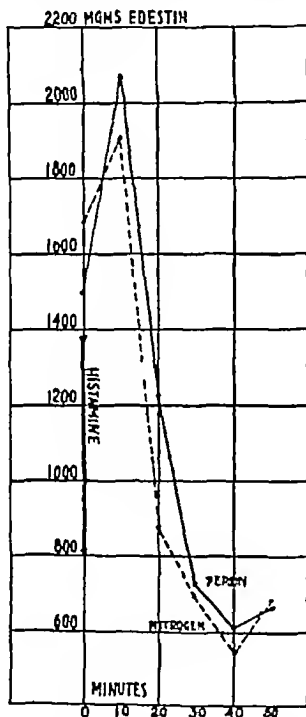


CHART 3 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 3 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

thermore, the gastric juice was unusually viscid and it was suspected that the nitrogen values would be high. The concentration of pepsin was distinctly lower than in cases 1, 2 and 3, but showed the usual drop

TABLE 4  
*Data from case 4*

| Volume of specimen | Appearance                               | Titratable acidity |       | Nitrogen concentration | Pepsin                                 |   |
|--------------------|--|--------------------|-------|------------------------|--|---|
|                    |  | Free               | Total |                        | Mgm edestin digested by 1 cc. of juice | Total mgm digested per 10 minute period |
| cc                 |  |                    |       | mgm per 100 cc         |  |   |
| 50                 | Bile stained, mucoid                     |                    |       |                        |  |   |
| 22                 | Thin fluid, slightly bile tinged, mucoid | 35                 | 50    | 75 0                   | 680                                    | 14,960                                  |

## Histamine 0.6 mgm

|    |  |     |     |      |     |        |
|----|--|-----|-----|------|-----|--------|
| 16 | Thin fluid, faint brownish (blood) tinge | 35  | 50  | 84 0 | 950 | 15,200 |
| 20 | Thin fluid, faint brownish (blood) tinge | 110 | 110 | 53 4 | 870 | 17,400 |
| 26 | Thin fluid, faint brownish (blood) tinge | 135 | 145 | 42 6 | 750 | 19,500 |
| 16 | Thin fluid, faint brownish (blood) tinge | 130 | 140 | 43 5 | 530 | 8,480  |
| 10 | Thin fluid, faint brownish (blood) tinge | 118 | 125 | 55 0 | 810 | 8,100  |

TABLE 5  
*Data from case 5*

| Volume of specimen | Appearance                                    | Titratable acidity |       | Nitrogen concentration | Pepsin                                 |   |
|--------------------|---|--------------------|-------|------------------------|--|---|
|                    |   | Free               | Total |                        | Mgm edestin digested by 1 cc. of juice | Total mgm digested per 10 minute period |
| cc                 |   |                    |       | mgm per 100 cc         |  |   |
| 30                 | Thin fluid, very slight bile tinge—some mucus |                    |       |                        |  |   |
| 22                 | Colorless fluid, some mucus                   | 94                 | 100   | 52 5                   | 365                                    | 8,030                                   |

## Histamine 0.6 mgm

|    |                   |     |     |      |     |        |
|----|-------------------|-----|-----|------|-----|--------|
| 26 | Water clear fluid | 86  | 93  | 29 4 | 405 | 10,530 |
| 38 | Water clear fluid | 119 | 124 | 43 5 | 595 | 22,610 |
| 55 | Water clear fluid | 126 | 130 | 35 1 | 350 | 19,250 |
| 45 | Water clear fluid | 128 | 133 | 28 5 | 345 | 15,525 |
| 35 | Water clear fluid | 118 | 125 | 27 9 | 290 | 10,150 |

after stimulation The curve also ran parallel with nitrogen concentration but when the nitrogen values were charted as in the previous

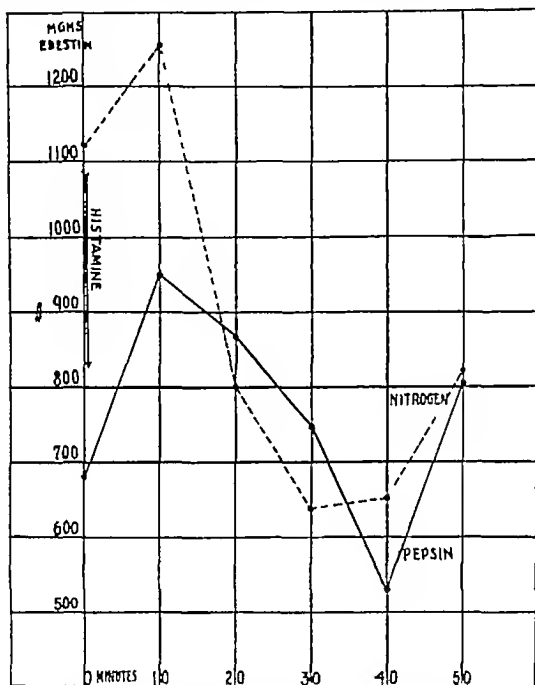


CHART 4 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 4 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

cases ( $N \text{ mgm per } 100 \text{ cc} \times 15$ ) a wide gap between the two curves is seen In other words, while a relation between nitrogen and pepsin seems likely there is relatively much more nitrogen present than usual

## DISCUSSION OF CASES 1-6

The first point which becomes evident from the preceding charts is the necessity of interpreting the concentration of pepsin in relation to the secretory activity of the stomach. Clearly, a determination of pepsin on a single specimen aspirated after a test meal gives only the most rudimentary sort of information. In case 3, for example, the digestive power of a cubic centimeter of juice was nearly 2100 mgm

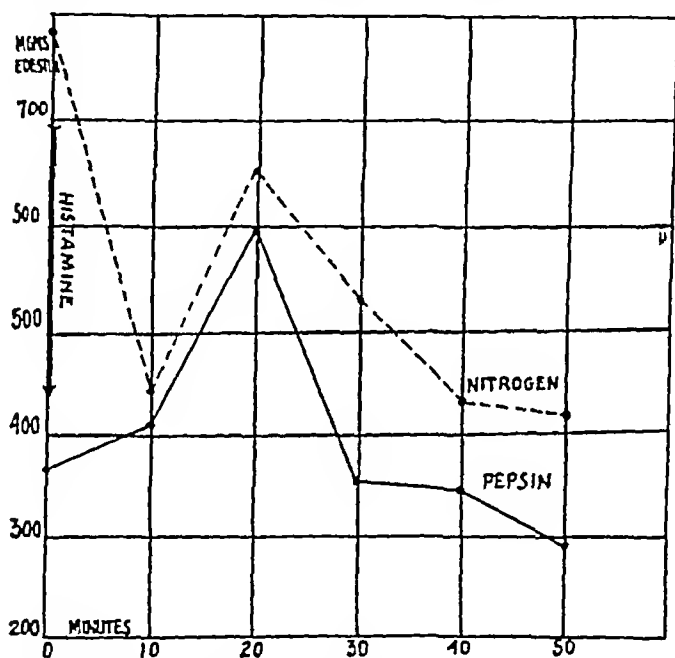


CHART 5 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 5 AT TEN-MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

of edestin 10 minutes after stimulation, 30 minutes later it was only 600 mgm. The most striking point in all the curves is the fall in concentration of pepsin at the height of secretion. This is to some extent explained by dilution from increased output of water at this time but as appears from chart 9 the total amount of pepsin secreted is actually increased. The actual values both for concentration and total output of pepsin vary considerably in the six cases. Many

more examinations would be necessary to define accurately normal standards but the limits are indicated.

The parallelism between the curves of pepsin and nitrogen concentration suggests strongly that pepsin is secreted in combination or

TABLE 6  
*Data from case 6*

| Volume of specimen | Appearance                  | Titratable acidity |       | Nitrogen concentration | Pepsin                                  |  |
|--------------------|-----------------------------|--------------------|-------|------------------------|---|--|
|                    |                             | Free               | Total |                        | Mgm. edestin digested by 1 cc. of juice | Total mgm. digested per 10 minute period |
| cc                 |                             |                    |       | mgm per 100 cc         |   |  |
| 70                 | Colorless fluid—very mucoid |                    |       |                        |   |  |
| 21                 | Colorless fluid—very mucoid | 8                  | 24    | 109                    | 685                                     | 14,385                                   |
| Histamine 0.5 mgm  |                             |                    |       |                        |   |  |
| 28                 | Colorless fluid—very mucoid | 22                 | 35    | 100                    | 690                                     | 19,320                                   |
| 40                 | Colorless fluid—very mucoid | 56                 | 68    | 116                    | 860                                     | 34,400                                   |
| 47                 | Colorless fluid—very mucoid | 63                 | 75    | 81.4                   | 610                                     | 28,670                                   |
| 50                 | Colorless fluid—very mucoid | 52                 | 63    | 86.0                   | 520                                     | 26,000                                   |
| 36                 | Colorless fluid—very mucoid | 64                 | 75    | 69.5                   | 450                                     | 16,200                                   |

TABLE 7  
*Relation of acidity to pepsin concentration of gastric juice*

| Case | Highest acidity | Highest concentration pepsin |
|------|-----------------|------------------------------|
| 4    | 135             | 870                          |
| 1    | 128             | 1,540                        |
| 5    | 128             | 595                          |
| 2    | 126             | 1,730                        |
| 3    | 121             | 2,075                        |
| 6    | 63              | 860                          |

in association with some nitrogenous body. It is equally clear that there is no close correlation between the acidity of the gastric juice and its peptic activity (see table 7)

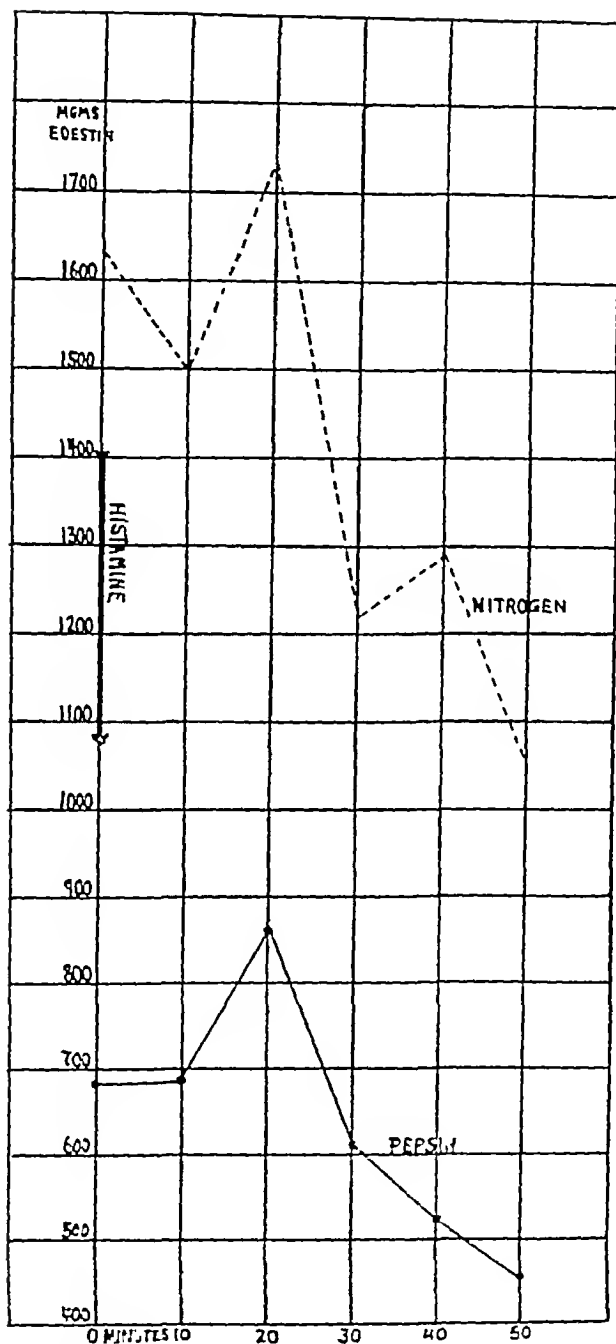


CHART 6 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CAST 6 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

TABLE 8  
*Data from case 7*

| Volume of specimen | Appearance                  | Titratable acidity |       | Nitrogen concentration | Pepsin                                  |  |
|--------------------|-----------------------------|--------------------|-------|------------------------|---|--|
|                    |                             | Free               | Total |                        | Mgm. edestin digested by 1 cc. of juice | Total mgm. digested per 10 minute period |
| cc                 |                             |                    |       | mgm per 100 cc.        |   |  |
| 40                 | Turbid colorless fluid      |                    |       |                        |   |  |
| 29                 | Colorless thin mucoid fluid | 12                 | 25    | 45.3                   | 380                                     | 11.020                                   |

Histamine 0.6 mgm.

|    |                   |    |    |      |     |       |
|----|-------------------|----|----|------|-----|-------|
| 13 | Water clear fluid | 27 | 37 | 32.1 | 318 | 4,111 |
| 17 | Water clear fluid | 43 | 53 | 43.5 | 270 | 4,624 |
| 16 | Water clear fluid | 60 | 70 | 50.1 | 320 | 4,960 |
| 15 | Water clear fluid | 27 | 40 | 54.9 | 170 | 2,580 |
| 7  | Water clear fluid | 23 | 41 | 47.3 | 265 | 1,955 |

TABLE 9  
*Data from case 8*

| Volume of specimen | Appearance                   | Titratable acidity |       | Nitrogen concentration | Pepsin                                  |  |
|--------------------|------------------------------|--------------------|-------|------------------------|---|--|
|                    |                              | Free               | Total |                        | Mgm. edestin digested by 1 cc. of juice | Total mgm. digested per 10 minute period |
| cc.                |                              |                    |       | mgm per 100 cc.        |   |  |
| 30                 | Clear colorless mucoid fluid |                    |       |                        |   |  |
| 7                  | Clear colorless mucoid fluid | 0                  |       | 104.6                  | 17                                      | 112                                      |

Histamine 0.6 mgm

|     |                              |   |  |       |    |     |
|-----|------------------------------|---|--|-------|----|-----|
| 4   | Clear colorless mucoid fluid | 0 |  | 105.6 | 60 | 240 |
| 3.5 | Clear colorless mucoid fluid | 0 |  | 126.6 | 50 | 175 |
| 3   | Clear colorless mucoid fluid | 0 |  | 142.0 | 85 | 240 |
| 3   | Clear colorless mucoid fluid | 0 |  | 137.3 | 50 | 150 |

## OBSERVATIONS IN GASTRIC DISORDERS

The following observations, made in patients with evidence of deficient gastric secretion, bring out certain points of importance in contrast to the preceding group

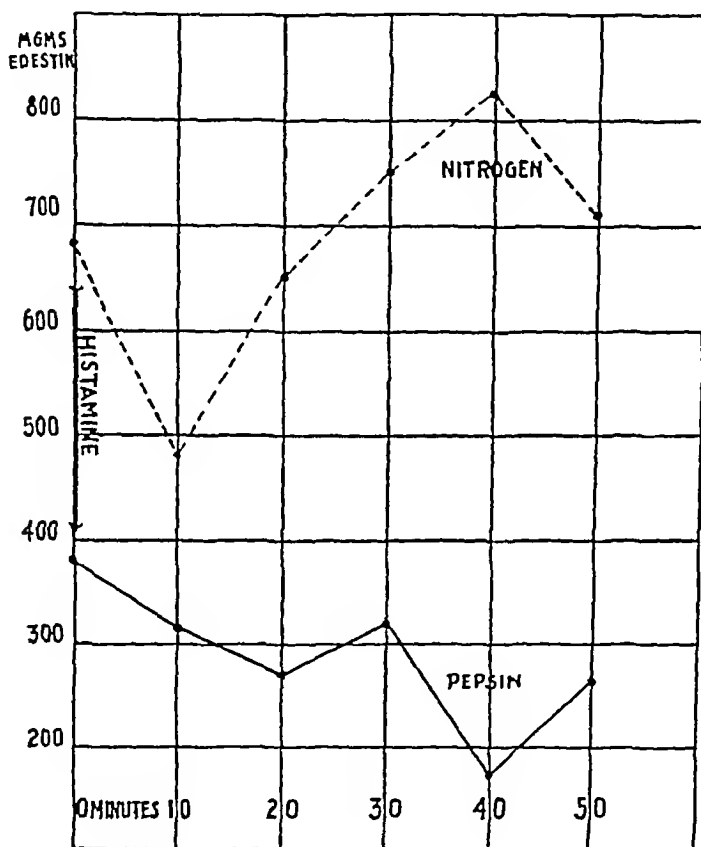


CHART 7 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 7 AT TEN-MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

*Case 7* A man, age 57, was under treatment in the Hospital for sprue. Routine fractional gastric analyses showed the presence of HCl in the stomach contents but only in small amount. The observations are summarized in table 8 and chart 7. The gastric acidity even after histamine stimulation is low but the volume of secretion is

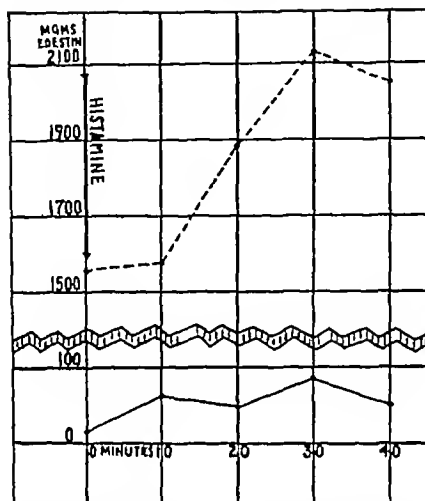


CHART 8 CURVES OF NITROGEN AND PEPSIN CONCENTRATION FROM CASE 8 AT TEN MINUTE INTERVALS BEFORE AND AFTER HISTAMINE

TABLE 10  
Data from case 9

| Volume of specimen | Appearance                                 | Titratable acidity |       | Nitrogen concentration | Pepsin                                  |  |
|--------------------|--|--------------------|-------|------------------------|---|--|
|                    |  | Free               | Total |                        | Mgm. edestin digested by 1 cc. of juice | Total mgm. digested per 10 minute period |
| cc.                |  |                    |       | mgm. per 100 cc        |   |  |
| 30                 | Fasting contents foul mucus, pus and blood | 0                  |       |                        |   |  |
| 5                  | Pale brown foul thin fluid                 | 0                  |       |                        | 58+                                     | 290+                                     |
| Histamine 0.5 mgm. |  |                    |       |                        |   |  |
| 5 5                | Pale brown foul thin fluid                 | 0                  |       |                        | 57                                      | 313                                      |
| 0 5                | Pale brown foul thin fluid                 | 0                  |       |                        | 20                                      | 80                                       |
| 3 5                | Pale grey brown mucoid, foul               | 0                  |       |                        |   |  |
| 0                  |  |                    |       |                        |   |  |

not remarkable The concentration of pepsin is much lower than in the normal cases and it neither shows the typical drop after stimulation

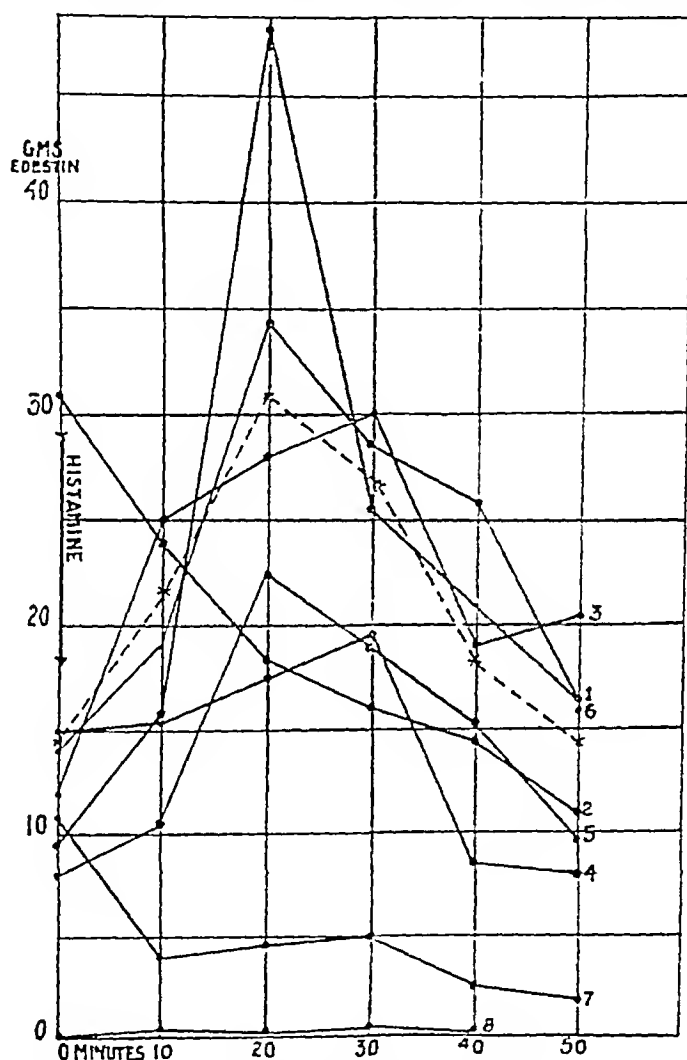


CHART 9 TOTAL OUTPUT OF PEPSIN PER TEN-MINUTE PERIOD BEFORE AND AFTER HISTAMINE

nor does it follow the nitrogen curve The total output of pepsin is also low

*Case 8* A woman, age 57, had been under observation for four years with pernicious anemia. The findings were typical and recently there had been a good response to liver feeding although at the time of the present test there was an anacidity even after histamine stimulation. The results are shown in table 9 and chart 8. The usual almost complete absence of secretion is evident. However, in spite of the anacidity the small amount of juice obtained showed definite though diminished peptic activity, the concentration of pepsin being roughly one tenth to one twenty-fifth normal. There was no relation between the nitrogen and pepsin curves and the total output of pepsin (chart 9) was, of course, small—approximately one hundredth normal.

*Case 9* A man, age 72, had an advanced cancer of the stomach with ascites and masses in the liver. When examined it was possible to obtain only small amounts (see table 10) of a brownish foul fluid containing considerable granular debris. The specimens were centrifuged and the supernatant fluid tested. The fluid itself contained a considerable amount of protein precipitable by trichloroacetic acid. This was measured and a correction was made. The table shows that even in the presence of an advanced sloughing carcinoma with total absence of acid, pepsin was still present in readily demonstrable amounts.

#### SUMMARY

A series of observations on the application in the clinic of a quantitative method of pepsin estimation are reported. The curves of pepsin concentration and of total pepsin output before and after stimulation by histamine are described. In normal people the concentration of pepsin falls markedly after stimulation and follows closely the curve of nitrogen concentration. The total output of pepsin is usually increased.

Aberrations from the normal are illustrated and it is pointed out that even in pernicious anemia of long standing and in advanced cancer of the stomach, some pepsin may still be secreted. Estimation of pepsin may turn out to be a more delicate index of gastric damage, in certain types of cases, than determinations of acidity.

## BIBLIOGRAPHY

- 1 Polland, W S , and Bloomfield, A L , J Clin Invest , 1929, vii, 45 A  
Quantitative Method for the Estimation of Pepsin
- 2 Polland, W S , Roberts, A M , and Bloomfield, A L , J Clin Invest , 1928,  
v, 611 The Chloride, Base and Nitrogen Content of Gastric Juice after  
Histamine Stimulation
- 3 Lim, R K S , Matheson, A R , and Schlapp, W , Quart J Exp Physiol ,  
1923, xii, 361 Observations on the Human Gastro-Duodenal Secretions  
with Special Reference to the Action of Histamine

# STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM<sup>1</sup>

## II THE CALCIUM EXCRETION OF NORMAL INDIVIDUALS ON A LOW CALCIUM DIET, ALSO DATA ON A CASE OF PREGNANCY<sup>2</sup>

BY WALTER BAUER,<sup>3</sup> FULLER ALBRIGHT,<sup>4</sup> AND JOSEPH C AUB

*(From the Medical Clinic of the Massachusetts General Hospital, Boston)*

(Received for publication September 14, 1928)

As a result of the lead studies carried on in this laboratory (1), (2), it has become increasingly apparent that it is important to be able to alter the deposition or mobilization of calcium phosphate in bone. A greater knowledge of the factors which influence calcium balance has therefore been sought. The decision as to the method of approaching this problem involved the following considerations. In the first place it was obvious that very little could be learned by studying the serum calcium alone, as this merely shows the height of the "calcium stream" but gives no indication as to the direction of its flow, into the excretory channels or into the bones. So it at once was clear that analyses of calcium intake and output would be a necessary addition to the blood studies. Furthermore, inasmuch as calcium is excreted into the bowel as well as into the urine, any figure obtained for fecal calcium would consist of two components, the calcium which had passed through the intestines unabsorbed and the calcium which had been absorbed and re-excreted. The latter of these alone can be thought of as actively taking part in the calcium metabolism. A way of overcoming this difficulty would be to have a diet with a calcium intake of zero but adequate in every other respect. Since this is not practicable we adopted a diet with a calcium intake as low

<sup>1</sup> Note —The title of this series has been changed from Studies of Inorganic Salt Metabolism to Studies of Calcium and Phosphorus Metabolism

<sup>2</sup> The expenses of this investigation were defrayed in part from the Lead Fund of Harvard University

<sup>3</sup> Medical Resident, Massachusetts General Hospital

<sup>4</sup> Research Fellow, Massachusetts General Hospital and Harvard Medical School

as possible and still adequate in caloric, protein, and vitamin requirements. By studying the calcium balance of an individual while on such a low calcium intake (about 100 mgm a day), one can deduce fairly well the rate of endogenous calcium exchange. A further advantage of such a technique is that, by having a very low calcium intake, one largely escapes the many variables which influence calcium *absorption*, such as the acid-base values of the diet (3), the amount of fat (4) (5) or of vitamin D in the diet, and the amount of exposure to ultra violet light. Therefore, a low calcium diet has been used in observing the calcium excretion of normal and pathological individuals, in order to determine factors which caused deviations from the normal. It is the purpose of this paper to present the data for the calcium excretion of some normal people.

#### NORMAL VARIABLES WHICH INFLUENCE CALCIUM METABOLISM

Other factors which might influence the calcium excretion are (a) sex, (b) age, (c) weight or body surface, (d) activity of subject, (e) acid-base values of diet, (f) amounts of other cations in intake, and (g) the phosphorus metabolism.

Sex plays a small but definite part in the quantitative comparison of the energy metabolism. Unless its effects upon calcium metabolism were of a relatively different magnitude it would be insignificant, because the methods available in determining the calcium metabolism are far less precise. In our studies it so happens that our control determinations are all made on men, whereas many of the pathological conditions studied were in women, so that this is a possible source of a small error in our comparisons.

Again, as in energy metabolism, it is not unlikely that age affects in some measure the rate of the calcium metabolism. Of course during the period of bone growth, there must be a marked quantitative effect on the calcium metabolism. In old age the atrophy of bone, as seen by x-ray, suggests a possible alteration in the rate of the calcium metabolism. Aside from these extremes of life, the basal metabolism remains quite constant and it is unlikely that there would be any marked variation in the rate of normal calcium exchange. Our control subjects varied between 19 years and 60 years with an average age of 41.3 years.

The size of the individual must play some part in the rate of the calcium metabolism. Sherman (6), in an effort to determine the calcium requirement for maintenance in man, found it useful to reduce his figures for the calcium excretion, under various inadequate intakes, to the calcium excretion per seventy kilograms. Possibly the calcium excretion per unit of surface area would be theoretically more correct, but we have followed the lead of Sherman and have reduced our calcium excretions and negative calcium balances in our controls to the amount per kilogram of body weight. However, for comparative purposes, we use the figures for the average calcium excretion per person per three day period without reference to weight, and then give the average weight of the control subjects.

As regards the activity of the subject and its effect on calcium metabolism, there is little known. In bone the phenomenon of atrophy of disuse is a very striking and real one, but as far as we know there have been no experiments to show whether this represents an altered calcium metabolism throughout, or merely in the immobilized parts of the body. Experiments are being planned to determine this fact. All but two of our controls were patients up and about the hospital ward, these two were doctors doing strenuous hospital work.

The ingestion of mineral acids has been proven to increase the calcium excretion (1) (7) (8) (9) (10) (11). Diets in which the mineral acid elements predominate over the fixed base elements also lead to decalcification (11) (12) (13) (14). Thus Bogert and Kirkpatrick (12) were able to increase the calcium excretion, especially that in the urine, by changing the food allowance of potato (a basic substance) to rice (an acid substance). The literature leads one to believe that an excess of alkali also leads to decalcification (15) (16). The modus operandi here, however, is possibly due chiefly to decreased absorption because of the increased alkalinity of the intestinal contents (3). For comparative studies like our own, therefore, the acid base balance of the diet should be constant even if not in acid-base equilibrium. We have not attempted to balance each diet in this respect, but have been content to use a very limited number of food substances, believing that, in this way, the acid base values would be fairly constant. In order to estimate the variation of our diets in this respect we have shown in table 1 and figure 1 the acid and base ele

as possible and still adequate in caloric, protein, and vitamin requirements. By studying the calcium balance of an individual while on such a low calcium intake (about 100 mgm a day), one can deduce fairly well the rate of endogenous calcium exchange. A further advantage of such a technique is that, by having a very low calcium intake, one largely escapes the many variables which influence calcium *absorption*, such as the acid-base values of the diet (3), the amount of fat (4) (5) or of vitamin D in the diet, and the amount of exposure to ultra violet light. Therefore, a low calcium diet has been used in observing the calcium excretion of normal and pathological individuals, in order to determine factors which caused deviations from the normal. It is the purpose of this paper to present the data for the calcium excretion of some normal people.

#### NORMAL VARIABLES WHICH INFLUENCE CALCIUM METABOLISM

Other factors which might influence the calcium excretion are (a) sex, (b) age, (c) weight or body surface, (d) activity of subject, (e) acid-base values of diet, (f) amounts of other cations in intake, and (g) the phosphorus metabolism.

Sex plays a small but definite part in the quantitative comparison of the energy metabolism. Unless its effects upon calcium metabolism were of a relatively different magnitude it would be insignificant, because the methods available in determining the calcium metabolism are far less precise. In our studies it so happens that our control determinations are all made on men, whereas many of the pathological conditions studied were in women, so that this is a possible source of a small error in our comparisons.

Again, as in energy metabolism, it is not unlikely that age affects in some measure the rate of the calcium metabolism. Of course during the period of bone growth, there must be a marked quantitative effect on the calcium metabolism. In old age the atrophy of bone, as seen by x-ray, suggests a possible alteration in the rate of the calcium metabolism. Aside from these extremes of life, the basal metabolism remains quite constant and it is unlikely that there would be any marked variation in the rate of normal calcium exchange. Our control subjects varied between 19 years and 60 years with an average age of 41.3 years.

(17) and Clark (18) The values in diets C, D, and E, except for sulphur and chlorine, were also checked by actual determination, so that the calculated and actually determined values can be compared. The value of each acid or base element was reduced to cubic centimeters of tenth normal solution, phosphoric acid being considered divalent (17). The total acid value thus obtained was then compared with the total base value and the acid or base balance value noted (see table 1 and figure 1). It will be seen that all five diets

TABLE 1  
*Acid-base properties of five typical diets†*

|                  | Diet A | Diet B | Diet C     |       | Diet D     |       | Diet E     |       |
|------------------|--------|--------|------------|-------|------------|-------|------------|-------|
|                  |        |        | Calculated | Found | Calculated | Found | Calculated | Found |
| Calcium          | 56     | 41     | 66         | 52    | 55         | 43    | 35         | 31    |
| Magnesium        | 230    | 168    | 215        |       | 196        |       | 118        |       |
| Potassium        | 774    | 533    | 882        |       | 781        |       | 446        |       |
| Sodium           | 495    | 419    | 525        |       | 400        |       | 312        |       |
| Total base       | 1,555  | 1,161  | 1,688      | 2,026 | 1,432      | 1,647 | 911        | 1,223 |
| Phosphorus*      | 762    | 453    | 735        | 600   | 530        | 440   | 375        | 369   |
| Chlorine         | 454    | 391    | 453        |       | 380        |       | 272        |       |
| Sulphur          | 679    | 424    | 793        |       | 557        |       | 387        |       |
| Total fixed acid | 1,895  | 1,268  | 1,981      |       | 1,467      |       | 1,034      |       |
| Excess of acid   | 340    | 107    | 293        |       | 35         |       | 123        |       |
| Calories         | 2,874  | 2,660  | 3,051      |       | 2,345      |       | 1,421      |       |

\* Calculated as divalent.

† All figures in cubic centimeters of N/10, except calories.

had an excess of acid over base—the most acid diet having a net acid value of 340 cc of tenth normal and the least acid a net acid value of 35 cc of tenth normal. These variations do not seem excessive. The calculated values agree fairly well with the actually determined values. The total base values actually determined are higher than the calculated ones, due probably to the amount of salt allowed in the diet.<sup>5</sup> The total acid-base metabolism is also

<sup>5</sup> The effect of acid and base excesses in the diet have been thoroughly studied and will be reported in a subsequent paper in this series.

ments of five diets Diets A and B are two of the actual diets used by two of the control patients, the patient eating diet A had the highest urinary calcium excretion of any of the control patients, and the patient eating diet B had one of the lowest urinary calcium ex-

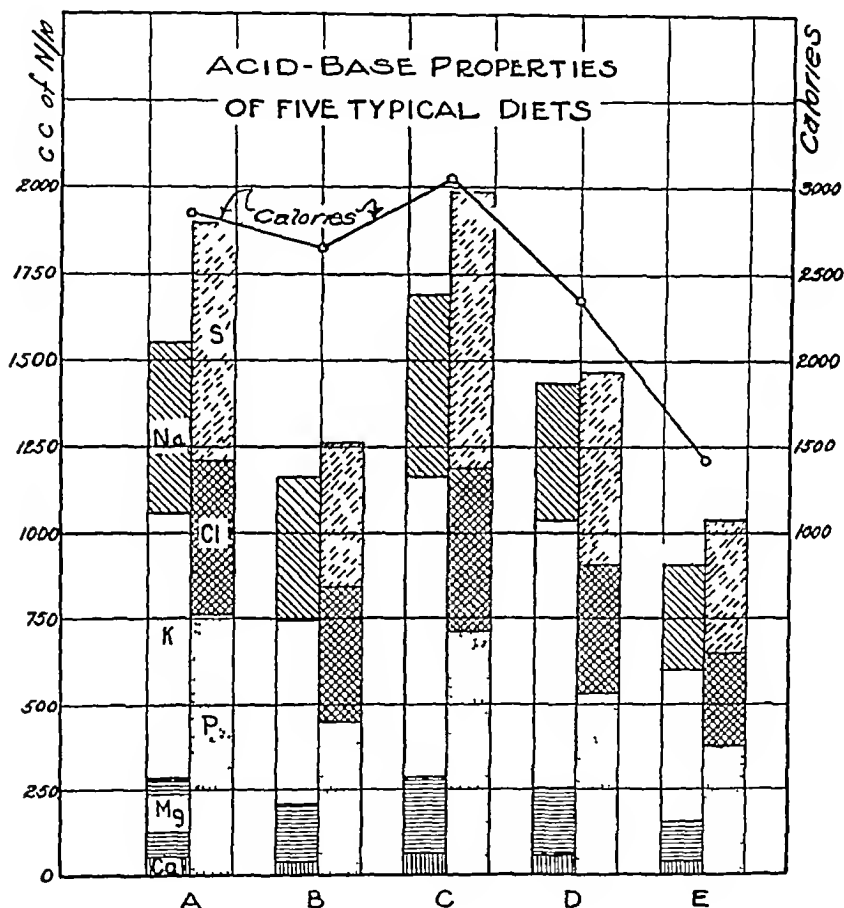


FIG 1

cretions Thus it was believed that here, if anywhere, we would find a discrepancy between the acid-base values of the diets

Diets C, D, and E happen to have been used in later experiments, though they are similar to those used in these controls The values for the acid and base elements are taken from Sherman and Gettler

doing hospital work. We have in all forty six three-day periods on these controls. No period was used unless the subject had had a fore period on a low calcium diet (about 100 mgm a day) of at least 36 hours, or unless at least three days had passed since any medication such as potassium iodide had been used. The details of the prepara-

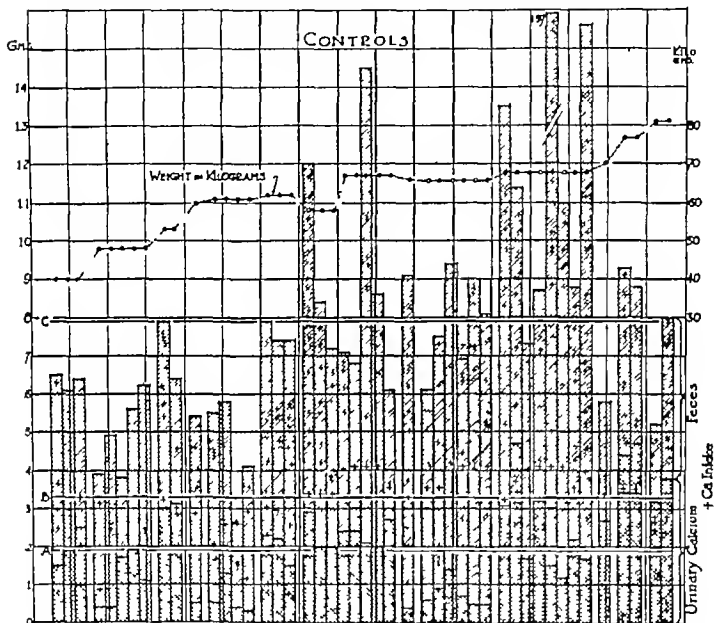


FIG 2 CALCIUM BALANCE OF 13 CONTROL SUBJECTS ON A LOW CALCIUM DIET

Line *A* represents average urinary calcium excretion, line *B* average calcium intake, line *C* average total calcium excretion. The distance *BC*, therefore, represents the average negative calcium balance.

tion of the diet, the collection of the excreta, and the methods of analysis have been given in a previous paper (24). The data are presented in table 2 and figure 2. The periods from each subject have been grouped together but they are not necessarily consecutive periods. The subjects themselves have been arranged in order of their weights.

dependent upon the nitrogen balance of the subject, for if the subject is in negative nitrogen balance, one should add to the acid-base values of the ingested food, the acid-base values of the tissue destroyed. Thus in every subject whose calcium metabolism is being determined, one should know the nitrogen balance. This we have done throughout our studies, except in our controls where only two were studied from this aspect. It may be said, however, that the controls were receiving more than a gram of protein per kilo per day, were not losing weight and therefore a negative nitrogen balance, if present, must have been small.

Because of the various inter-relations which the cations, calcium, magnesium, sodium and potassium have (14) (19) (20) (21) (22), in a study of any one of them, it would be desirable that the intakes of all the others be absolutely fixed. To what extent this has been the case in five of our diets can be seen by again referring to table 1 and figure 1.

Calcium metabolism is inseparably linked with phosphorus metabolism. Most of the calcium retained in the body is usually assumed to be in the form of tertiary calcium phosphate deposited in the bones. Phosphoric acid (ingested either as such or as the acid phosphate) produces marked decalcification (11). Neutral sodium phosphate injected into the blood stream also causes an increased calcium excretion (23). So, under ideal conditions, quantitative calcium metabolism studies, if to be used for comparison with other experiments, should have a constant phosphorus intake. We have not attempted to do this but have been content with the variations which our small menu allowed. Table 1 and figure 1 show the actual variation found in five of our diets. As in the discussion of the acid-base values of the diet, it must be pointed out here that unless the subject is in nitrogen equilibrium, the phosphorus in the diet really consists of the phosphorus ingested plus the phosphorus liberated from the destruction of body protein.

#### EXPERIMENTS

Studies have been made on thirteen individuals. Eleven of these were convalescing from industrial lead poisoning, but were otherwise normal. We have no reason to suppose that lead poisoning affects the rate of calcium metabolism. The other two subjects were doctors

TABLE 2—Continued

| Subject | Age  | Weight      | Period | Calcium in grams per 3-day period |       |                 |        |         | Total calcium excretion per kilogram | Negative calcium balance per kilogram |
|---------|------|-------------|--------|-----------------------------------|-------|-----------------|--------|---------|--------------------------------------|---------------------------------------|
|         |      |             |        | Urine                             | Feces | Total excretion | Intake | Balance |                                      |                                       |
|         |      | <i>Kgm.</i> |        |                                   |       |                 |        |         | <i>grams</i>                         | <i>grams</i>                          |
| D M     | 50   | 66          | 6      | 0 06                              | 0 55  | 0 61            | 0 355  | -0 25   | 0 012                                | 0 0060                                |
|         |      |             | 7      | 0 09                              | 0 66  | 0 75            | 0 355  | -0 39   |                                      |                                       |
|         |      |             | 14     | 0 14                              | 0 79  | 0 93            | 0 420  | -0 51   |                                      |                                       |
|         |      |             | 15     | 0 07                              | 0 62  | 0 69            | 0 411  | -0 28   |                                      |                                       |
|         |      |             | 16     | 0 05                              | 0 85  | 0 90            | 0 405  | -0 49   |                                      |                                       |
| J T     | 33   | 68          | 17     | 0 05                              | 0 76  | 0 81            | 0 348  | -0 46   | 0 018                                | 0 0119                                |
|         |      |             | 9      | 0 31                              | 1 04  | 1 35            | 0 326  | -1 01   |                                      |                                       |
|         |      |             | 18     | 0 47                              | 0 67  | 1 14            | 0 372  | -0 77   |                                      |                                       |
|         |      |             | 41     | 0 17                              | 0 56  | 0 73            | 0 405  | -0 33   |                                      |                                       |
|         |      |             | 42     | 0 32                              | 0 55  | 0 87            | 0 411  | -0 46   |                                      |                                       |
|         |      |             | 43     | 0 15                              | 1 80  | 1 95            | 0 411  | -1 54   |                                      |                                       |
|         |      |             | 44     | 0 12                              | 0 98  | 1 10            | 0 411  | -0 69   |                                      |                                       |
| J M     | 35   | 70          | 53     | 0 22                              | 0 66  | 0 88            | 0 357  | -0 52   | 0 008                                | 0 0034                                |
|         |      |             | 59     | 0 17                              | 1 39  | 1 56            | 0 399  | -1 16   |                                      |                                       |
| C H.    | 25   | 77          | 2      | 0 27                              | 0 31  | 0 58            | 0 339  | -0 24   | 0 012                                | 0 0071                                |
|         |      |             | 1      | 0 44                              | 0 48  | 0 92            | 0 35   | -0 57   |                                      |                                       |
| C K.    | 30   | 81 4        | 2      | 0 47                              | 0 41  | 0 88            | 0 35   | -0 53   | 0 008                                | 0 0052                                |
|         |      |             | 1      | 0 32                              | 0 20  | 0 52            | 0 28   | -0 24   |                                      |                                       |
| Average | 42 3 | 67 2        | 2      | 0 38                              | 0 46  | 0 84            | 0 23   | -0 61   | 0 012                                | 0 0067                                |
|         |      |             | 1      | 0 19                              | 0 60  | 0 79            | 0 33   | -0 46   |                                      |                                       |

## RESULTS

It will be seen from table 2 and figure 2 that there is considerable variation in the calcium excretion not only among different individuals but also among different periods of the same individual. This variation could probably be reduced by a more rigid standardization of the aforementioned normal factors which influence calcium metabolism. However, these figures give us an indication of the amount of calcium which a normal person will excrete on a low calcium diet and their average will be used for future comparisons. Attention

TABLE 2  
Calcium studies in "normal" controls

| Subject | Age | Weight     | Period | Calcium in grams per 3-day period |       |                 |        |         | Total calcium excretion per kilogram | Negative calcium balance per kilogram |
|---------|-----|------------|--------|-----------------------------------|-------|-----------------|--------|---------|--------------------------------------|---------------------------------------|
|         |     |            |        | Urine                             | Feces | Total excretion | Intake | Balance |                                      |                                       |
|         |     | <i>kem</i> |        |                                   |       |                 |        |         | <i>grams</i>                         | <i>grams</i>                          |
| J P     | 60  | 40         | 20     | 0 15                              | 0 50  | 0 65            | 0 299  | -0 35   | 0 016                                | 0 0081                                |
|         |     |            | 5      | 0 19                              | 0 42  | 0 61            | 0 290  | -0 32   |                                      |                                       |
|         |     |            | 9      | 0 25                              | 0 39  | 0 64            | 0 336  | -0 30   |                                      |                                       |
| W C     | 19  | 48         | 2      | 0 04                              | 0 35  | 0 39            | 0 085  | -0 30   | 0 010                                | 0 0056                                |
|         |     |            | 3      | 0 04                              | 0 44  | 0 49            | 0 246  | -0 24   |                                      |                                       |
|         |     |            | 4      | 0 17                              | 0 21  | 0 38            | 0 246  | -0 13   |                                      |                                       |
|         |     |            | 5      | 0 19                              | 0 37  | 0 56            | 0 246  | -0 31   |                                      |                                       |
|         |     |            | 6      | 0 11                              | 0 51  | 0 62            | 0 246  | -0 37   |                                      |                                       |
| M R     | 53  | 53         | 5      | 0 30                              | 0 49  | 0 79            | 0 322  | -0 47   | 0 013                                | 0 0076                                |
|         |     |            | 6      | 0 31                              | 0 33  | 0 64            | 0 300  | -0 34   |                                      |                                       |
| N D     | 55  | 60         | 13     | 0 05                              | 0 49  | 0 54            | 0 288  | -0 25   | 0 009                                | 0 004                                 |
| K K     | 53  | 61         | 6      | 0 05                              | 0 50  | 0 55            | 0 208  | -0 34   | 0 007                                | 0 0032                                |
|         |     |            | 7      | 0 13                              | 0 45  | 0 58            | 0 258  | -0 32   |                                      |                                       |
|         |     |            | 26     | 0 04                              | 0 22  | 0 26            | 0 262  | 0 0     |                                      |                                       |
|         |     |            | 27     | 0 03                              | 0 38  | 0 41            | 0 294  | -0 12   |                                      |                                       |
| M C     | 50  | 62         | 22     | 0 23                              | 0 56  | 0 79            | 0 303  | -0 49   | 0 012                                | 0 0076                                |
|         |     |            | 23     | 0 22                              | 0 52  | 0 74            | 0 298  | -0 44   |                                      |                                       |
|         |     |            | 11     | 0 15                              | 0 59  | 0 74            | 0 252  | -0 49   |                                      |                                       |
| P M     | 48  | 67         | 31     | 0 29                              | 0 81  | 1 10            | 0 340  | -0 76   | 0 014                                | 0 0078                                |
|         |     |            | 39     | 0 20                              | 0 65  | 0 85            | 0 340  | -0 51   |                                      |                                       |
|         |     |            | 40     | 0 20                              | 0 52  | 0 72            | 0 340  | -0 38   |                                      |                                       |
|         |     |            | 63     | 0 24                              | 0 47  | 0 71            | 0 405  | -0 31   |                                      |                                       |
|         |     |            | 64     | 0 24                              | 0 44  | 0 68            | 0 411  | -0 27   |                                      |                                       |
|         |     |            | 65     | 0 21                              | 1 24  | 1 45            | 0 411  | -1 04   |                                      |                                       |
|         |     |            | 66     | 0 20                              | 0 66  | 0 86            | 0 411  | -0 45   |                                      |                                       |
| P S     | 39  | 64         | 75     | 0 27                              | 0 44  | 0 71            | 0 357  | -0 25   | 0 014                                | 0 0091                                |
|         |     |            | 34     | 0 04                              | 0 86  | 0 91            | 0 330  | -0 58   |                                      |                                       |

TABLE 2—Continued

| Subject | Age  | Weight<br>kgm | Period | Calcium in grams per 3-day period |       |                 |        |         | Total<br>calcium<br>excretion<br>per<br>kilogram | Negative<br>calcium<br>balance<br>per<br>kilogram |
|---------|------|---------------|--------|-----------------------------------|-------|-----------------|--------|---------|--|---|
|         |      |               |        | Urine                             | Feces | Total excretion | Intake | Balance |  |   |
| D M     | 50   | 66            | 6      | 0 06                              | 0 55  | 0 61            | 0 355  | -0 25   | 0 012  | 0 0060  |
|         |      |               | 7      | 0 09                              | 0 66  | 0 75            | 0 355  | -0 39   |  |   |
|         |      |               | 14     | 0 14                              | 0 79  | 0 93            | 0 420  | -0 51   |  |   |
|         |      |               | 15     | 0 07                              | 0 62  | 0 69            | 0 411  | -0 28   |  |   |
|         |      |               | 16     | 0 05                              | 0 85  | 0 90            | 0 405  | -0 49   |  |   |
|         |      |               | 17     | 0 05                              | 0 76  | 0 81            | 0 348  | -0 46   |  |   |
| J T     | 33   | 68            | 9      | 0 31                              | 1 04  | 1 35            | 0 326  | -1 01   | 0 018  | 0 0119  |
|         |      |               | 18     | 0 47                              | 0 67  | 1 14            | 0 372  | -0 77   |  |   |
|         |      |               | 41     | 0 17                              | 0 56  | 0 73            | 0 405  | -0 33   |  |   |
|         |      |               | 42     | 0 32                              | 0 55  | 0 87            | 0 411  | -0 46   |  |   |
|         |      |               | 43     | 0 15                              | 1 80  | 1 95            | 0 411  | -1 54   |  |   |
|         |      |               | 44     | 0 12                              | 0 98  | 1 10            | 0 411  | -0 69   |  |   |
| J M     | 35   | 70            | 53     | 0 22                              | 0 66  | 0 88            | 0 357  | -0 52   | 0 008  | 0 0034  |
|         |      |               | 59     | 0 17                              | 1 39  | 1 56            | 0 399  | -1 16   |  |   |
| C H.    | 25   | 77            | 2      | 0 27                              | 0 31  | 0 58            | 0 339  | -0 24   | 0 012  | 0 0071  |
|         |      |               | 1      | 0 44                              | 0 48  | 0 92            | 0 35   | -0 57   |  |   |
| C K.    | 30   | 81 4          | 2      | 0 47                              | 0 41  | 0 88            | 0 35   | -0 53   | 0 008  | 0 0052  |
|         |      |               | 1      | 0 32                              | 0 20  | 0 52            | 0 28   | -0 24   |  |   |
| Average | 42 3 | 67 2          |        | 0 19                              | 0 60  | 0 79            | 0 33   | -0 46   | 0 012  | 0 0067  |

## RESULTS

It will be seen from table 2 and figure 2 that there is considerable variation in the calcium excretion not only among different individuals but also among different periods of the same individual. This variation could probably be reduced by a more rigid standardization of the aforementioned normal factors which influence calcium metabolism. However, these figures gave us an indication of the amount of calcium which a normal person will excrete on a low calcium diet and their average will be used for future comparisons. Attention

TABLE 3

*Calcium studies on low calcium diets (taken from the literature)*

| Author                           | Weight    | Calcium in grams per 3-day period   |   |   |   |  | Total calcium excretion per kilogram |
|----------------------------------|-----------|---|---|---|---|--|--------------------------------------|
|                                  |           | Urine   | Feces   | Total excretion   | Intake  | Balance  |                                      |
| Von Wendt (36)                   | kgm<br>71 | 0 090   | 0 468   | 0 558   | 0 147   | -0 411   | grams<br>0 0079                      |
| Sherman, Wheeler and Yates (31)  | 57        | 0 240<br>0 330<br>0 360<br>0 390<br>0 450<br>0 390<br>0 360                   | 0 600<br>0 360<br>0 360<br>0 270<br>0 360<br>0 450<br>0 330                   | 0 840<br>0 690<br>0 720<br>0 660<br>0 810<br>0 840<br>0 690                   | 0 420<br>0 450<br>0 420<br>0 420<br>0 390<br>0 390<br>0 390                   | -0 420<br>-0 240<br>-0 300<br>-0 240<br>-0 420<br>-0 450<br>-0 300                     |                                      |
| Average                          |           | 0 360   | 0 390   | 0 750   | 0 411   | -0 339   | 0 0132                               |
| Sherman and Winters (32)         | 54        | 0 180<br>0 150<br>0 180<br>0 180<br>0 270<br>0 270<br>0 200<br>0 300<br>0 240 | 0 450<br>0 540<br>0 540<br>0 450<br>0 570<br>0 750<br>0 750<br>1 080<br>1 020 | 0 630<br>0 690<br>0 720<br>0 630<br>0 840<br>1 020<br>0 950<br>1 380<br>1 260 | 0 240<br>0 240<br>0 240<br>0 240<br>0 600<br>0 600<br>0 600<br>0 990<br>0 990 | -0 390<br>-0 450<br>-0 480<br>-0 390<br>-0 240<br>-0 420<br>-0 350<br>-0 390<br>-0 270 |                                      |
| Average                          |           | 0 219   | 0 683   | 0 902   | 0 527   | -0 375   | 0 0167                               |
| Sherman (6)                      | 80        | 0 330<br>0 510<br>0 480<br>0 540<br>0 540                                     | 0 630<br>0 450<br>0 450<br>0 480<br>0 480                                     | 0 960<br>0 960<br>0 930<br>1 020<br>1 020                                     | 0 690<br>0 630<br>0 600<br>0 630<br>0 630                                     | -0 270<br>-0 330<br>-0 330<br>-0 390<br>-0 390   |                                      |
| Average                          |           | 0 480   | 0 498   | 0 978   | 0 636   | -0 342   | 0 0122                               |
| Sherman, Wheeler, and Yates (31) | 54        | 0 300<br>0 390<br>0 420<br>0 390<br>0 540<br>0 570<br>0 510                   | 0 750<br>0 570<br>0 540<br>0 570<br>0 540<br>0 570<br>0 600                   | 1 050<br>0 960<br>0 960<br>0 960<br>1 080<br>1 140<br>1 110                   | 0 750<br>0 780<br>0 780<br>0 750<br>0 750<br>0 750<br>0 750                   | -0 300<br>-0 180<br>-0 180<br>-0 210<br>-0 330<br>-0 390<br>-0 360                     |                                      |
| Average                          |           | 0 446   | 0 591   | 1 037   | 0 759   | -0 278   | 0 0192                               |

TABLE 3—Continued

| Author                            | Weight      | Calcium in grams per 3-day period |       |                 |        |         | Total calcium excretion per kilogram |
|-----------------------------------|-------------|-----------------------------------|-------|-----------------|--------|---------|--------------------------------------|
|                                   |             | Urine                             | Feces | Total excretion | Intake | Balance |                                      |
|                                   | <i>kgm.</i> |                                   |       |                 |        |         | <i>grams</i>                         |
| Rose (37)                         | 45.5        | 0 174                             | 0 594 | 0 768           | 0 846  | +0 078  |                                      |
|                                   |             | 0 225                             | 0 375 | 0 600           | 0 759  | +0 159  |                                      |
|                                   |             | 0 243                             | 0 651 | 0 894           | 0 747  | -0 147  |                                      |
| Average                           |             | 0 214                             | 0 540 | 0 754           | 0 784  | +0 030  | 0 0166                               |
| Sherman, Gillett, and Pope (30)   | 50          | 0 210                             | 0 600 | 0 810           | 0 810  | 0 000   |                                      |
|                                   |             | 0 270                             | 0 840 | 1 110           | 0 810  | -0 300  |                                      |
|                                   |             | 0 300                             | 0 840 | 1 140           | 0 840  | -0 300  |                                      |
|                                   |             | 0 360                             | 0 870 | 1 230           | 0 810  | -0 420  |                                      |
|                                   |             | 0 360                             | 0 810 | 1 170           | 0 810  | -0 360  |                                      |
|                                   |             | 0 360                             | 0 780 | 1 140           | 0 810  | -0 330  |                                      |
|                                   |             | 0 360                             | 0 870 | 1 230           | 0 840  | -0 390  |                                      |
|                                   |             |                                   |       |                 |        |         |                                      |
| Average                           |             | 0 317                             | 0 801 | 1 118           | 0 818  | -0 300  | 0 0224                               |
| Sherman, Gillett, and Pope (30)   | 57          | 0 330                             | 0 660 | 0 990           | 0 810  | -0 180  |                                      |
|                                   |             | 0 410                             | 0 630 | 1 040           | 0 810  | -0 230  |                                      |
|                                   |             | 0 410                             | 0 690 | 1 100           | 0 840  | -0 260  |                                      |
|                                   |             | 0 370                             | 0 450 | 0 820           | 0 840  | +0 020  |                                      |
|                                   |             | 0 360                             | 0 660 | 1 020           | 0 840  | -0 180  |                                      |
|                                   |             | 0 370                             | 0 480 | 0 850           | 0 840  | -0 010  |                                      |
|                                   |             | 0 440                             | 0 570 | 1 010           | 0 840  | -0 170  |                                      |
|                                   |             | 0 340                             | 0 570 | 0 910           | 0 840  | -0 070  |                                      |
|                                   |             |                                   |       |                 |        |         |                                      |
| Average                           |             | 0 379                             | 0 589 | 0 968           | 0 833  | -0 135  | 0 0170                               |
| Sherman Winters and Phillips (33) | 54          | 0 125                             | 0 795 | 0 920           | 0 570  | -0 350  |                                      |
|                                   |             | 0 285                             | 1 065 | 1 350           | 0 930  | -0 420  |                                      |
|                                   |             | 0 420                             | 0 810 | 1 230           | 0 930  | -0 300  |                                      |
|                                   |             | 0 330                             | 0 930 | 1 260           | 0 930  | -0 330  |                                      |
| Average                           |             | 0 290                             | 0 900 | 1 190           | 0 840  | -0 350  | 0 0220                               |
| Sherman, Gillett and Pope (30)    | 52          | 0 320                             | 0 840 | 1 160           | 0 900  | -0 260  |                                      |
|                                   |             | 0 340                             | 0 840 | 1 180           | 0 900  | -0 280  |                                      |
|                                   |             | 0 390                             | 0 810 | 1 200           | 0 870  | -0 330  |                                      |
|                                   |             | 0 340                             | 1 170 | 1 510           | 0 870  | -0 640  |                                      |
|                                   |             | 0 270                             | 0 870 | 1 140           | 0 870  | -0 270  |                                      |
|                                   |             | 0 260                             | 0 870 | 1 130           | 0 870  | -0 260  |                                      |
|                                   |             | 0 250                             | 0 570 | 0 820           | 0 900  | +0 080  |                                      |
|                                   |             | 0 270                             | 0 750 | 1 020           | 0 900  | -0 120  |                                      |
|                                   |             | 0 260                             | 0 900 | 1 160           | 0 900  | -0 260  |                                      |
|                                   |             | 0 270                             | 0 750 | 1 020           | 0 900  | -0 120  |                                      |
|                                   |             |                                   |       |                 |        |         |                                      |
| Average                           |             | 0 291                             | 0 837 | 1 134           | 0 888  | -0 246  | 0 0218                               |

TABLE 3—Continued

| Author                              | Weight     | Calcium in grams per 3-day period |       |                 |        |         | Total calcium excretion per kilogram |
|-------------------------------------|------------|-----------------------------------|-------|-----------------|--------|---------|--------------------------------------|
|                                     |            | Urine                             | Feces | Total excretion | Intake | Balance |                                      |
|                                     | <i>kgm</i> |                                   |       |                 |        |         | <i>grams</i>                         |
| Rose (37)                           | 48         | 0 249                             | 0 498 | 0 747           | 0 771  | +0 024  |                                      |
|                                     |            | 0 186                             | 0 480 | 0 666           | 0 963  | +0 297  |                                      |
|                                     |            | 0 150                             | 0 390 | 0 540           | 0 963  | +0 423  |                                      |
| Average                             |            | 0 195                             | 0 456 | 0 651           | 0 899  | +0 248  | 0 0136                               |
| Sherman, Winters, and Phillips (33) | 67         | 0 180                             | 1 170 | 1 350           | 0 720  | -0 630  |                                      |
|                                     |            | 0 390                             | 0 930 | 1 320           | 0 720  | -0 600  |                                      |
|                                     |            | 0 315                             | 0 840 | 1 155           | 0 720  | -0 435  |                                      |
|                                     |            | 0 510                             | 1 125 | 1 635           | 1 050  | -0 585  |                                      |
|                                     |            | 0 420                             | 1 050 | 1 470           | 1 050  | -0 420  |                                      |
|                                     |            | 0 450                             | 1 290 | 1 740           | 1 050  | -0 690  |                                      |
|                                     |            | 0 405                             | 1 050 | 1 455           | 1 050  | -0 405  |                                      |
|                                     |            |                                   |       |                 |        |         |                                      |
| Average                             |            | 0 381                             | 1 065 | 1 446           | 0 908  | -0 538  | 0 0216                               |
| Sherman (6)                         | 69         | 1 020                             | 0 240 | 1 260           | 0 540  | -0 720  |                                      |
|                                     |            | 0 840                             | 0 330 | 1 170           | 1 170  | 0 000   |                                      |
|                                     |            | 1 080                             | 0 670 | 1 750           | 1 200  | -0 550  |                                      |
| Average                             |            | 0 980                             | 0 413 | 1 393           | 0 970  | -0 423  | 0 0202                               |
| Rose (37)                           | 56         | 0 210                             | 0 792 | 1 002           | 1 149  | +0 147  |                                      |
|                                     |            | 0 207                             | 0 726 | 0 933           | 1 149  | +0 216  |                                      |
|                                     |            | 0 207                             | 0 573 | 0 780           | 1 149  | +0 369  |                                      |
|                                     |            | 0 222                             | 0 861 | 1 083           | 0 945  | -0 138  |                                      |
|                                     |            | 0 231                             | 0 567 | 0 798           | 0 945  | +0 147  |                                      |
|                                     |            | 0 219                             | 0 981 | 1 200           | 0 945  | -0 255  |                                      |
|                                     |            | 0 216                             | 0 660 | 0 876           | 0 945  | +0 069  |                                      |
|                                     |            | 0 237                             | 0 861 | 1 098           | 0 945  | -0 153  |                                      |
|                                     |            |                                   |       |                 |        |         |                                      |
| Average                             |            | 0 218                             | 0 753 | 0 971           | 1 021  | +0 050  | 0 0173                               |
| Bogert and Kirkpatrick (12)         | 53         | 0 447                             | 0 744 | 1 191           | 1 023  | -0 168  | 0 0225                               |
| Rose (37)                           | 54         | 0 243                             | 0 870 | 1 113           | 1 149  | +0 036  |                                      |
|                                     |            | 0 213                             | 0 729 | 0 942           | 1 149  | +0 207  |                                      |
|                                     |            | 0 207                             | 0 645 | 0 852           | 1 149  | +0 297  |                                      |
|                                     |            | 0 168                             | 0 801 | 0 969           | 1 149  | +0 180  |                                      |
|                                     |            | 0 099                             | 0 399 | 0 498           | 0 945  | +0 447  |                                      |
|                                     |            | 0 135                             | 0 495 | 0 630           | 0 945  | +0 315  |                                      |
|                                     |            | 0 228                             | 0 708 | 0 936           | 0 945  | +0 009  |                                      |
|                                     |            | 0 228                             | 0 705 | 0 933           | 0 945  | +0 012  |                                      |
|                                     |            | 0 180                             | 0 717 | 0 897           | 0 945  | +0 048  |                                      |
|                                     |            |                                   |       |                 |        |         |                                      |
|                                     |            |                                   |       |                 |        |         |                                      |
| Average                             |            | 0 189                             | 0 674 | 0 863           | 1 036  | +0 173  | 0 0160                               |

TABLE 3—*Concluded*

| Author                      | Weight     | Calcium in grams per 3-day period |       |                 |        |         | Total calcium excretion per kilogram |
|-----------------------------|------------|-----------------------------------|-------|-----------------|--------|---------|--------------------------------------|
|                             |            | Urine                             | Feces | Total excretion | Intake | Balance |                                      |
|                             | <i>kgm</i> |                                   |       |                 |        |         | <i>grams</i>                         |
| Sherman (6)                 | 61         | 0 450                             | 1 260 | 1 710           | 1 380  | -0 330  |                                      |
|                             |            | 0 540                             | 1 500 | 2 040           | 1 230  | -0 810  |                                      |
|                             |            | 0 600                             | 0 780 | 1 380           | 1 230  | -0 150  |                                      |
|                             |            | 0 600                             | 1 020 | 1 620           | 1 260  | -0 360  |                                      |
|                             |            | 0 600                             | 0 960 | 1 560           | 0 960  | -0 600  |                                      |
|                             |            | 0 600                             | 0 630 | 1 230           | 0 960  | -0 270  |                                      |
| Average                     |            | 0 565                             | 1 025 | 1 590           | 1 170  | -0 420  | 0 0260                               |
| Bogert and Kirkpatrick (12) | 64         | 0 306                             | 0 984 | 1 290           | 1 185  | -0 105  | 0 0201                               |
| Bogert and Kirkpatrick (12) | 70         | 0 393                             | 1 152 | 1 545           | 1 185  | -0 360  | 0 0221                               |
| Final average               | 56 6       | 0 343                             | 0 710 | 1 053           | 0 831  | -0 222  | 0 0185                               |

tion is called to the fact that only one individual was in calcium balance and that individual for only one period. The group had an average intake of 0.33 gram per three days, an average output in the urine of 0.19 gram, in the feces of 0.60 gram, making a total average output of 0.79 gram resulting in an average negative calcium balance of 0.46 gram.

In order to compare our results with other similar experiments taken from the literature, tables 3 and 4 and figure 3 have been constructed. In table 3 are given figures for total calcium studies similar to ours, except that different food substances were used in many cases and that the calcium intakes were usually greater. The subjects here are arranged in order of increasing calcium intake rather than by weight. We have recalculated the figures of these other investigators in order that they may be comparable to our figures, i.e., all figures have been reduced to grams of calcium per individual per three day period. In table 4 are given the data for the calcium excretions of fasting men. These observations differ from those in which the diet is inadequate in calcium only (*vide supra*) because of the great drain on body tissues. In figure 3 these data from the literature are given in graphic form for comparison with the values from figure 2, the average of which is

TABLE 4  
*Calcium studies on "fasting men" (taken from the literature)*

| Author                                 | Weight  | Calcium in grams per 3-day period |       |                 |                 |         | Total calcium excretion per kilogram | Negative nitrogen balance per 3 days |
|--|---------|-----------------------------------|-------|-----------------|-----------------|---------|--------------------------------------|--------------------------------------|
|  |         | Urine                             | Feces | Total excretion | Intake in water | Balance |                                      |                                      |
|  | kgm     |                                   |       |                 |                 |         | grams                                | grams                                |
| Benedict (38)                          | 58      | 1 020                             | 0 000 | 1 020           | 0 000           | -1 020  |                                      | 31 6                                 |
|  |         | 1 086                             | 0 000 | 1 086           | 0 000           | -1 086  |                                      | 30 8                                 |
|  |         | 0 950                             | 0 000 | 0 950           | 0 000           | -0 950  |                                      | 30 7                                 |
|  |         | 0 913                             | 0 000 | 0 913           | 0 000           | -0 913  |                                      | 30 6                                 |
|  | 52      | 0 957                             | 0 000 | 0 957           | 0 000           | -0 957  |                                      | 28 1                                 |
|  |         | 0 998                             | 0 000 | 0 998           | 0 000           | -0 998  |                                      | 25 8                                 |
|  |         | 0 914                             | 0 000 | 0 914           | 0 000           | -0 914  |                                      | 23 1                                 |
|  |         | 0 718                             | 0 000 | 0 718           | 0 000           | -0 718  |                                      | 23 5                                 |
|  | 49      | 0 611                             | 0 000 | 0 611           | 0 000           | -0 611  |                                      | 23 5                                 |
|  | 47      | 0 568                             | 0 000 | 0 568           | 0 000           | -0 568  |                                      | 22 5                                 |
|  | Average | 52                                | 0 873 | 0 000           | 0 873           | 0 000   | -0 873                               | 0 0163                               |
| Cathcart (39)                          | 60      | 0 552                             | 0 000 | 0 552           | 0 000           | -0 552  |                                      | 31 0                                 |
|  |         | 0 459                             | 0 000 | 0 459           | 0 000           | -0 459  |                                      | 25 0                                 |
|  |         | 0 288                             | 0 000 | 0 288           | 0 000           | -0 288  |                                      | 25 0                                 |
| Average                                | 60      | 0 433                             | 0 000 | 0 433           | 0 000           | -0 433  | 0 0072                               | 27 0                                 |
| Mueller, Munk, Senator, and Zuntz (40) | 57      | 0 885                             | 0 208 | 1 093           | 0 171           | -0 922  |                                      | 38 1                                 |
|  |         | 1 070                             | 0 208 | 1 278           | 0 171           | -1 107  |                                      | 31 7                                 |
| Average                                | 57      | 0 977                             | 0 208 | 1 185           | 0 171           | -1 014  | 0 0208*                              | 35 0                                 |
| Mueller, Munk, Senator, and Zuntz (40) | 60      | 0 305                             | 0 094 | 0 399           | 0 219           | -0 180  | 0 0067                               | 33 2                                 |
| Final average                          | 58      | 0 769                             | 0 031 | 0 800           | 0 035           | -0 765  | 0 0137                               | 28 4                                 |

\* Weight before fast, used

TABLE 5  
*Summary chart*

|                           | Number of 3-day periods | Weight     | Values for calcium in grams per 3-day period |       |       |                 |         | Total calcium excretion per kilogram | Negative nitrogen balance per 3 days |
|---------------------------|-------------------------|------------|--|-------|-------|-----------------|---------|--------------------------------------|--------------------------------------|
|                           |                         |            | Intake                                       | Urine | Feces | Total excretion | Balance |                                      |                                      |
|                           |                         | <i>kgm</i> |  |       |       |                 |         | <i>grams</i>                         | <i>grams</i>                         |
| Average values of table 2 | 46                      | 67 2       | 0 33   | 0 19  | 0 60  | 0 79            | -0 46   | 0 0120                               | —                                    |
| Average values of table 3 | 100                     | 56 6       | 0 83   | 0 35  | 0 71  | 1 05            | -0 22   | 0 0185                               | —                                    |
| Average values of table 4 | 16                      | 58         | 0 04   | 0 77  | 0 03  | 0 80            | -0 76   | 0 0137                               | 28 4                                 |

represented by the first column at the left in figure 3. A line separates the "fasting men" experiments from the others, as they are not quite comparable. In figure 3, aside from the "fasting men," each of whose

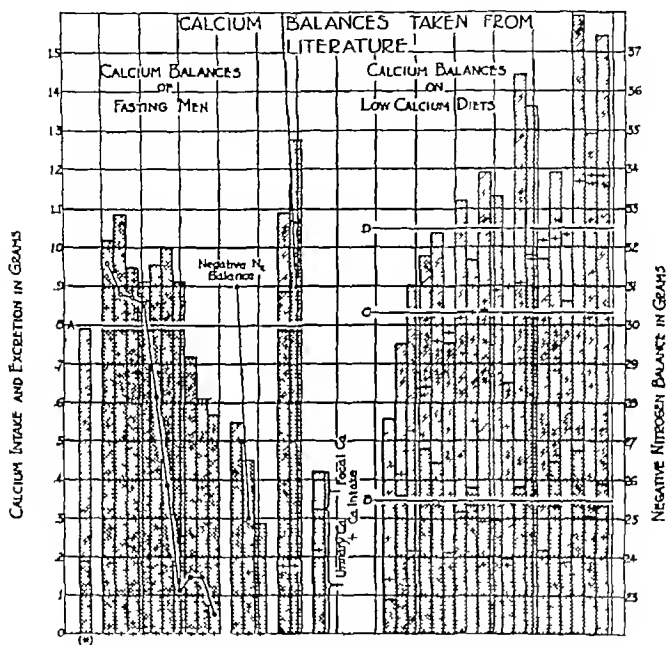


FIG. 3. CALCIUM BALANCES TAKEN FROM LITERATURE

A, Average urinary excretion of "fasting men" B, average urinary excretion of persons on a low calcium diet C, average calcium intake D, average total calcium output Negative nitrogen balances in the "fasting men" are represented thus o—o

\* Graphic representation of average calcium balance from our control series

three-day periods are charted separately only the average value for each individual is charted

In table 5 are given the average values of tables 2, 3, and 4. It

will be noted that the urinary calcium excretion is greater in the group from the literature than in our group. This is ascribed to the higher calcium intake. It has been stated that the urinary calcium increases on a low calcium diet (25). This was probably due to some variation in the diet other than the calcium, such as the acid-base value (v supra). This is borne out by the "fasting men" experiments, whose diet may be thought of as consisting of their own flesh, and therefore very acid. Their average negative nitrogen balance per three days of 28.4 grams would correspond to a calcium content of 0.067 gram (using Katz' figure for calcium value of human flesh) (26). Thus on this very low "intake" of calcium they had a very high average calcium excretion in the urine, 0.767 gram per three-day period.

#### DISCUSSION

From an inspection of our data and the data collected from the literature it is seen that there is a certain very appreciable minimal requirement of calcium necessary to keep the body in calcium balance. The literature gives abundant proof of this both by experiments on animals (8) (15) (27) (28) and by experiments on people (6) (29) (30) (31) (32) (33). It is this negative calcium balance on a very low calcium intake which we wish especially to emphasize, and the varying degree of which under certain abnormal conditions we intend to make the subject of later papers. We believe that this is an aspect of calcium metabolism which can be quantitatively determined. This appreciable excretion of a necessary body ingredient is not applicable to all body elements. Thus chlorine excretion during starvation sinks to almost zero and a diet deficient in chlorides does not lead to dechlorinization. Of direct interest in this connection are the experiments of Osborne and Mendel (27). These investigators have shown that rats continue to gain weight on diets very low in magnesium, sodium, chlorine or potassium. However, when sodium and potassium were both very low or when either calcium or phosphorus was very low, the rats ceased to gain. Furthermore, an excess of magnesium was without avail in making up for the calcium deficiency. It is their belief that sodium, potassium, magnesium and chlorine can be "husbanded" in the body, but that calcium and phosphorus and

at least one of the monovalent cations (sodium or potassium) must be furnished in certain minimal quantities. Likewise Hamilton (28), from calcium balance studies on premature infants, concluded that a certain definite amount of calcium had to be excreted each day regardless of the intake and that only when calcium was furnished in excess of this amount was there a positive balance.

The significance of this negative calcium balance on a low calcium diet can only be speculated on. Hamilton (28) suggests that the calcium may be necessary to neutralize acids. Osborne and Mendel (27) offer a similar explanation for the cessation of growth in their rats on diets very low in both sodium and potassium. A second possibility suggests itself. McCrudden (41) has shown that bone is constantly undergoing anabolism and catabolism. Is it possible that the calcium liberated in the process of catabolism is not available for anabolism but must be excreted? The negative calcium balance on a zero calcium intake might then be thought of as an index of the endogenous calcium metabolism. Experiments such as the ones now reported would thus be comparable to the early ones of Voit on nitrogen metabolism wherein the endogenous nitrogen metabolism was obtained by determining the nitrogen excretion during starvation (34) (39).

As a first step in the investigation of the cause of this negative calcium balance on a low calcium diet, it seemed of interest to see whether this relatively large amount of calcium ordinarily excreted on a very low calcium intake could be used during pregnancy to meet the fetal demands for calcium. Therefore, a young woman was put on a low calcium diet for three three-day periods during the fifth and eighth months of pregnancy and six weeks after delivery by Caesarian section. The findings are given in graphic form in figure 4. Some high calcium periods following the second and third group of low calcium intake periods are also charted, but will not be commented on here except to point out the rise in urinary calcium when the subject changed from a low calcium intake to a high calcium intake. It will be noted that this subject excreted practically the same amount of calcium in all three groups of low calcium periods, regardless of whether she was supplying a small amount of calcium to the fetus as in the fifth month of pregnancy,

a large amount as in the eighth month of pregnancy, or none at all as during the second month after delivery, which was also one

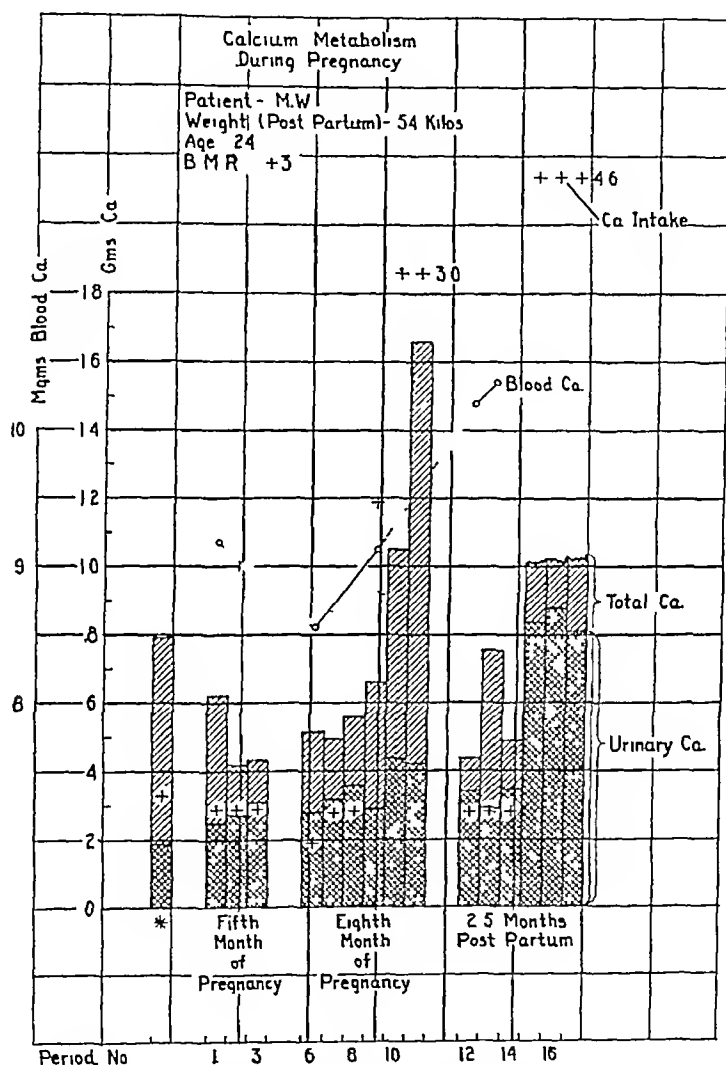


FIG 4 CALCIUM METABOLISM DURING PREGNANCY

\* Average calcium metabolism values of control series

month after cessation of lactation Furthermore, during all three periods of low calcium intake, this subject excreted about the same

amount of calcium as she would have been expected to do had she not been pregnant, as judged from the average excretion of our normal controls. Attention is called to the fact that this subject's non-pregnant weight is 54 kgm (average of controls 62 kgm) and that her sex is opposite to that of the controls.

The serum calcium and phosphorus values of this patient are of interest. The serum calcium is confirmatory of the work of Widdows who found a low calcium level during pregnancy (35).

This experiment suggests that the calcium excreted on a low calcium diet is not available for the fetus, just as it is not available during growth for the building of bones (28).

#### SUMMARY

1 Figures showing the negative calcium balances in 46 three-day periods in thirteen normal individuals on a very low calcium intake are recorded.

2 It is pointed out that before the negative calcium balance of two normal individuals on a low calcium diet can be compared, factors should be introduced to equalize differences in sex, age, surface area, activity, acid-base properties of diet, values of other cations in diet, and phosphorus metabolism. It is further pointed out that unless two such individuals are in nitrogen equilibrium, further factors have to be introduced to offset the total acid value and the phosphoric acid content liberated during the destruction of the body protein.

3 Our results are compared with similar but not identical experiments from the literature.

4 It is emphasized that the negative calcium balance on a low calcium diet is an aspect of calcium metabolism which can be studied quantitatively under varying conditions.

5 The cause of the appreciable negative calcium balance on a low calcium diet is discussed.

6 Observations on a pregnant woman are included which tend to show that the calcium excreted on a low calcium diet is not available for the fetus. The calcium excretion during gestation is essentially normal.

## BIBLIOGRAPHY

- 1 Aub, J C , Fairhall, L T , Minot, A S , and Reznikoff, P , *Medicine*, 1925, iv, 1    Lead Poisoning
- 2 Hunter, D , and Aub, J C , *Quart J Med* , 1927, xx, 123    Lead Studies XV The Effect of the Parathyroid Hormone on the Excretion of Lead and of Calcium in Patients Suffering from Lead Poisoning
- 3 Hamilton, B , and Moriarty, M , *Am J Dis Child* , 1928, xxxvi, 450    Factors Influencing the Excretion of Calcium
- 4 Telfer, S V , *Quart J Med* , 1926, xx, 1    Studies in Calcium and Phosphorus Metabolism IV The Influence of Free Fatty Acids in the Intestine on the Absorption and Excretion of the Mineral Elements
- 5 Telfer, S V , *Quart J Med* , 1926, xx, 7    Studies in Calcium and Phosphorus Metabolism V Infantile Rickets The Excretion and Absorption of the Mineral Elements and the Influence of Fats in the Diet on Mineral Absorption
- 6 Sherman, H C , *J Biol Chem* , 1920, xlv, 21    Calcium Requirement of Maintenance in Man
- 7 Stehle, R I , *J Biol Chem* , 1917, xxxi, 461    A Study of the Effect of Hydrochloric Acid on the Mineral Excretion of Dogs
- 8 Givens, M H , and Mendel, L B , *J Biol Chem* , 1917, xxxi, 421    Studies in Calcium and Magnesium Metabolism I The Effects of Base and Acid
- 9 Givens, M H , *J Biol Chem* , 1918, xxxv, 241    Studies in Calcium and Magnesium Metabolism V Further Observations on the Effect of Acid and other Dietary Factors
- 10 Goto, K , *J Biol Chem* , 1918, xxxvi, 355    Mineral Metabolism in Experimental Acidosis
- 11 Berg, R , *Biochem Ztschr* , 1910, xxx, 107    Über die Ausscheidung von per os eingeführten Phosphaten, besonders der Calciumphosphate
- 12 Bogert, L J , and Kirkpatrick, E E , *J Biol Chem* , 1922, lv, 375    Studies in Inorganic Metabolism II The Effects of Acid Forming and Base Forming Diets upon Calcium Metabolism
- 13 Givens, M H , *J Biol Chem* , 1917, xxxi, 441    Studies in Calcium and Magnesium Metabolism III The Effect of Fat and Fatty Acid Derivatives
- 14 Ferner, P , *Compt rend Acad d sc* , 1907, cxlv, 95    Calcification et decalcification chez l'homme
- 15 Steenbock, H , and Hart, E B , *J Biol Chem* , 1913, xlv, 59    The Influence of Function on the Lime Requirements of Animals
- 16 Sato, A , *Am J Dis Child* , 1918, xvi, 293    The Effect of Alkali and Malt Preparations upon the Retention of Calcium in Infancy
- 17 Sherman, H C , and Gettler, A O , *J Biol Chem* , 1912, xi, 323    The Balance of Acid-Forming and Base-Forming Elements in Foods, and its Relation to Ammonia Metabolism.

- 18 Clark, G W, J Biol Chem, 1925, lrv, 597 Acid and Base Forming Elements in Foods
- 19 Novi, J, Zentralbl f Biochem u Biophys, 1912, xlii, 578 Entkalkende Wirkung des Natriumchlorids in physiologischen Lösungen
- 20 Hellwig, L, Ztschr f Biol, 1921, lxxvi, 281 Chem Abstracts, 1922, xvi, 118 Gedanken über die knochenkalklösende Wirkung starken Kochsalzgenußes.
- 21 Emmerich, R, and Loew, O, Berl Klin Wchnschr, 1913, l, 1200 Der Einfluss der Kalksalze auf Konstitution und Gesundheit.
- 22 Hart, E B, and Steenbock, H., J Biol. Chem., 1913, xiv, 75 (Quoted by Sherman.) The Effect of a High Magnesium Intake on the Calcium Retention by Swine
- 23 Greenwald, L, and Gross, J, J Biol Chem., 1925, lxxvi, 201 The Excretion of Calcium, Phosphorus and Magnesium after the Injection of Calcium Chloride, Sodium Phosphate, or Both
- 24 Bauer, W, and Aub, J C, J Am. Dietet. Ass, 1927, lii, 106 Studies of Inorganic Salt Metabolism, I. The Ward Routine and Methods
- 25 Boekelman, W A., and Staal, J P, Arch f Exper Path u. Pharm., 1907, lvi, 260 (Quoted by Givens) Zur Kenntnis der Kalkausscheidung im Harn
- 26 Katz, J, Arch f d ges Physiol., 1896, lxvi, 1 (Quoted by Lusk.) Die mineralischen Bestandtheile des Muskelfleisches.
- 27 Osborne, T B, and Mendel, L. B, J Biol Chem., 1918, xxxiv, 131 The Inorganic Elements in Nutrition.
- 28 Hamilton, B, Acta Pediat., 1922, ii, 1 The Calcium and Phosphorus Metabolism of Prematurely Born Infants
- 29 Sherman, H C., Mettler, A J, and Sinclair, J E., U S Dept. Agric., Office of Experiment Stations, Bull. 227, 1910 Calcium, Magnesium and Phosphorus in Food and Nutrition
- 30 Sherman, H C., Gillett, L H, and Pope, H M, J Biol. Chem, 1918, xxxiv, 373 Monthly Metabolism of Nitrogen, Phosphorus and Calcium in Healthy Women.
- 31 Sherman, H C., Wheeler, L., and Yates, A B, J Biol. Chem., 1918, xxxiv, 383 Experiments on the Nutritive Value of Maize Protein and on the Phosphorus and Calcium Requirements of Healthy Women
- 32 Sherman, H C, and Winters, J C, J Biol. Chem, 1918, xxxv, 301 Efficiency of Maize Protein in Adult Human Nutrition.
- 33 Sherman, H C, Winters, J C., and Phillips, V, J Biol Chem, 1919, xxxix, 53 Efficiency of Oat Protein in Adult Human Nutrition
- 34 Voit, Hermann's Handbuch "Stoffwechsel," 1881, vi, Pt 1, p 106, 330, and 344 (Quoted by Lusk.)
- 35 Widdows S T, Biochem J, 1923, xvii, 34 Calcium Content of the Blood During Pregnancy

- 36 Von Wendt, G , Skandin Arch f Physiol , 1905, xvii, 211 Untersuchungen über den Eiweiss- und Salz-Stoffwechsel beim Menschen
- 37 Rose, M S , J Biol Chem , 1920, xli, 349 Experiments on the Utilization of the Calcium of Carrots by Man
- 38 Benedict, F G , A Study of Prolonged Fasting Carnegie Institute of Washington, (1915), Publication 203
- 39 Cathcart, E P , Biochem Ztschr , 1907, vi, 109 Über die Zusammensetzung des Hungerharns
- 40 Lehmann, C , Mueller, F , Munk, I , Senator, H , and Zuntz, N , Virchow's Archives, 1893, cxxxi, Supplement, p 168 Untersuchungen auf zwei hungernden Menschen
- 41 McCrudden, F H Endocrinology and Metabolism D Appleton and Co , 1922, Volume IV, 734

# STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

## III THE EFFECTS OF THE THYROID HORMONE AND THYROID DISEASE<sup>1</sup>

By JOSEPH C. AUB, WALTER BAUER,<sup>2</sup> CLARK HEATH<sup>3</sup> AND MARION ROPES

*(From the Medical Clinic of the Massachusetts General Hospital Boston)*

(Received for publication September 14, 1928)

### INTRODUCTION AND REVIEW OF THE LITERATURE

It is known that the internal secretion of the thyroid gland raises the basal rate of combustion of carbohydrate, fat and possibly protein, and consequently appreciably increases the total heat production of the organism, but its effect on calcium and phosphorus metabolism has not been definitely determined.

Studies of the effect of thyroid secretion on the calcium metabolism have been reported in the literature, but they are inconclusive because unsatisfactory methods were employed and because the data obtained were scanty and contradictory. A short summary of these observations, however, is of interest in this discussion as a background for our experiments on the relation of the thyroid gland to calcium and phosphorus metabolism. As early as 1892, Koeppen (1) reported that there appeared to be a connection between exophthalmic goiter and osteomalacia and other bone diseases. Pierallini (2) in 1906 investigated the possibility of an intimate relation between thyroid function and the metabolism of calcium and phosphorus. In his experiments urinary calcium only was determined, the calcium intake was unknown and unrestricted because he assumed that the urinary content is an index of endogenous calcium metabolism. These assumptions are now known to be incorrect so that his results are of limited value. His figures showed the calcium and phosphorus ex-

<sup>1</sup> The expenses of this investigation were defrayed in part from the Lead Fund of Harvard University.

<sup>2</sup> Medical Resident, Massachusetts General Hospital.

<sup>3</sup> Medical Intern, Massachusetts General Hospital.

cretions to be normal in Basedow's disease. Another article published in the same year by Scholz (3) gave the results of careful studies of the metabolism of cretins before and after thyroid therapy. The untreated cretin was found to retain phosphorus and to excrete an abnormally large quantity of alkaline earths. Administration of thyroid preparations exerted no marked influence on the phosphorus metabolism, but reduced the urinary excretion of the alkaline earths, particularly that of calcium. The fecal calcium, however, was increased. A year later, Silvestri and Tosatti (4) reported the effect of administration of thyroid extract on the calcium exchange in various diseases. They found that daily ingestion of "one tablet" of thyroid favored retention of calcium. The calcium output was determined in one-day periods during the first six days of thyroid medication, so that their results can probably be explained by insufficient dosage of thyroid and inadequate duration of observation. In referring to work done with Bolaffio and Tedesco, Falta (5) in 1909 mentioned experiments in which administration of thyroid increased the ratio of nitrogen to phosphoric acid in the urine and increased the phosphate in the stools, and concluded that there must be an increase in the fecal excretion of calcium to account for the augmented phosphorus output in the stools. Another series of observations on the effect of thyroid feeding on calcium excretion was made by Parhon (6) in 1912. In this very interesting study the calcium exchange of nine rabbits was determined both before and after administration of thyroid. The first group of animals received 0.05 gram of thyroid daily and suffered a net loss of 0.007 gram of calcium per kilo per week, the second group of three, all of which died within seventeen days, received 0.10 gram of thyroid daily and lost 0.228 gram (average figures) of calcium per week, the last three, which died within five days after the beginning of medication, received 0.3 gram of thyroid daily and lost on the average 0.662 gram of calcium per kilogram per week. These experiments indicate that the thyroid does exert a stimulating influence on calcium excretion. Kojima (7) in 1917 approached the problem from a different angle. He removed the thyroid gland and the parathyroid from rats and then replaced the latter. He then determined the nitrogen and calcium excretions and found that both had been diminished. He could not, however, demonstrate in these experiments that adminis-

tion of thyroid affected the calcium metabolism. The report included no data concerning the adequate functioning of the parathyroid transplants and therefore the reduced calcium excretion after roidectomy might be explained by parathyroid deficiency. Summer (8) in the same year published data obtained from observation for thirteen days of a patient with exophthalmic goiter. Two and one half liters of milk were given daily, which meant that the calcium intake was approximately 5 grams per day. No determinations were made of the basal metabolic rate and control calcium curves apparently were taken from the literature. The author concluded that the mineral excretion was high but very irregular, both calcium and phosphorus losses being especially great. Because the quantity of calcium in the urine was normal and that in the feces was large, he attributed the high fecal calcium in Basedow's disease to a difficulty in absorption rather than an abnormal excretion. Tins (9) stated, without data, in his book on the relation of the parathyroid glands to disease, that calcium is lost from the body during diabetes mellitus and Graves' disease and retained by myxedematous individuals.

In view of the uncontroverted importance of the thyroid secretion on calcium metabolism and the disagreement in the findings of previous authors, it seemed desirable to reinvestigate this subject.

## EXPERIMENTS

We have studied the calcium, phosphorus and nitrogen excretion in a series of patients with various thyroid diseases. The calcium excreted by an individual while on a diet adequate in all respects except in calcium should represent chiefly the endogenous calcium metabolism. Our results demonstrate conclusively that increased thyroid secretion caused a striking accentuation of the calcium excretion and likewise increased phosphorus and nitrogen elimination.

The methods used have been described in a previous communication (10). The daily diets were practically identical for each individual and except for their very low calcium content were well balanced and adequate. There was, however, a temporary negative nitrogen balance in the patients with exophthalmic goiter. The low calcium diet was a free choice diet which not only permits a more ac-

curate evaluation of the endogenous calcium metabolism, but it also largely eliminates the unknown factor of unabsorbed calcium found in the feces

## RESULTS

### *Serum calcium*

In this series of 14 cases it was found that the serum calcium and phosphorus values remained within the accepted normal limits, though a few cases suggested a slight fall in these values during the period of observation. The serum findings gave no indication of the rate of calcium metabolism, as may be seen when the data on calcium and phosphorus excretion are examined

### *Calcium excretion*

*1 In exophthalmic goiter* Our normal standards for calcium excretion have been obtained from thirteen normal individuals who were maintained upon a similar diet inadequate in calcium. These data are to be found in Paper II of this series

The most obvious conclusions which can be drawn from our data are the marked increase of calcium excretion in exophthalmic goiter and hyperfunctioning adenomata of the thyroid, and the striking decrease found in myxedema. (See table 4 and chart 1) This increase is out of proportion to the increase in the basal metabolic rate, for though the latter averages only 55 per cent above the normal in our six cases of exophthalmic goiter, the average calcium excretion is 170 per cent above our average control figures. These results are even more striking than they appear, for the average normal calcium excretion of 0.79 gram per three day period is based on the total excretion of men of average weight of 62 kgm, while the thyroid patients were thin individuals, some of them women, with an average weight of 50 kgm. When calculated for three day periods, the average calcium excretion for the normal individual becomes 12.7 mgm per kilogram of body weight, and for the patients with exophthalmic goiter 42.0 mgm per kilogram of body weight, which is an increase of 231 per cent above normal. This increase in calcium excretion is shown most strikingly in the case of Norman G. (chart 4), where 4.6 grams of calcium were



quate calcium intake on our routine diet, this increased calcium excretion must represent largely calcium loss from the body

2 *In toxic adenomata* Added evidence of the effect of thyroid on calcium metabolism was furnished by two typical cases with hyper-functioning thyroid adenomata in which the basal metabolic rates were not as high as in those suffering from exophthalmic goiter The

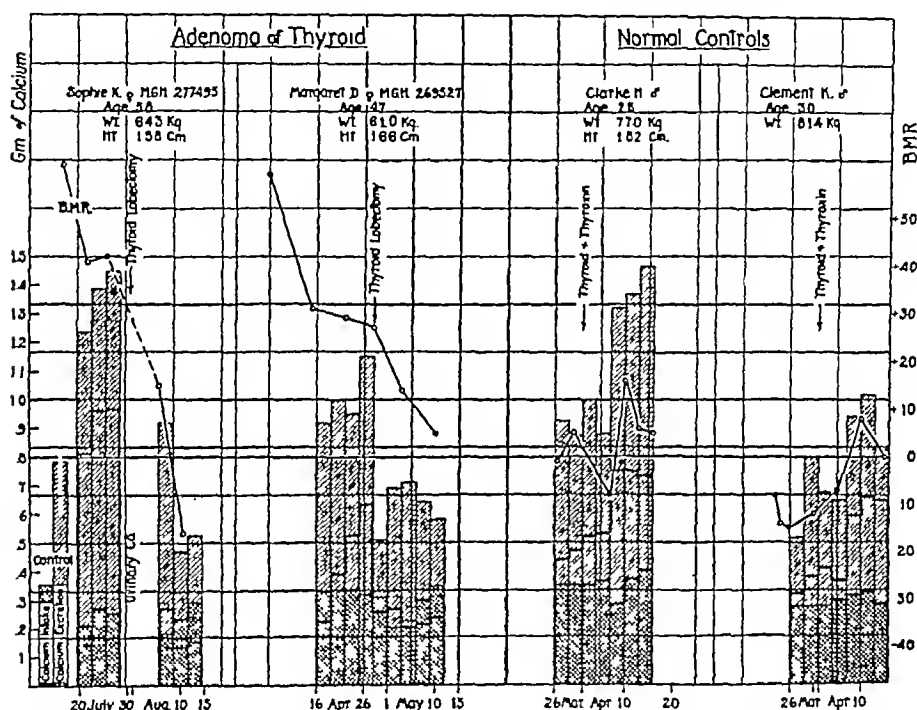


CHART 2 A GRAPHIC REPRESENTATION OF THE CALCIUM METABOLISM DATA SHOWN IN TABLES 5 AND 7

The calcium intake, urinary calcium excretion and fecal calcium excretion are represented in the same manner as shown in chart 1

calcium, phosphorus and nitrogen excretions were likewise less elevated than in the series of patients with Graves' disease (See table 5) With this small number, however, it is impossible to say that there is any fundamental difference in the calcium excretion in the two diseases The calcium excretion in these two cases was above normal and fell definitely after the adenomata were removed (chart 2)

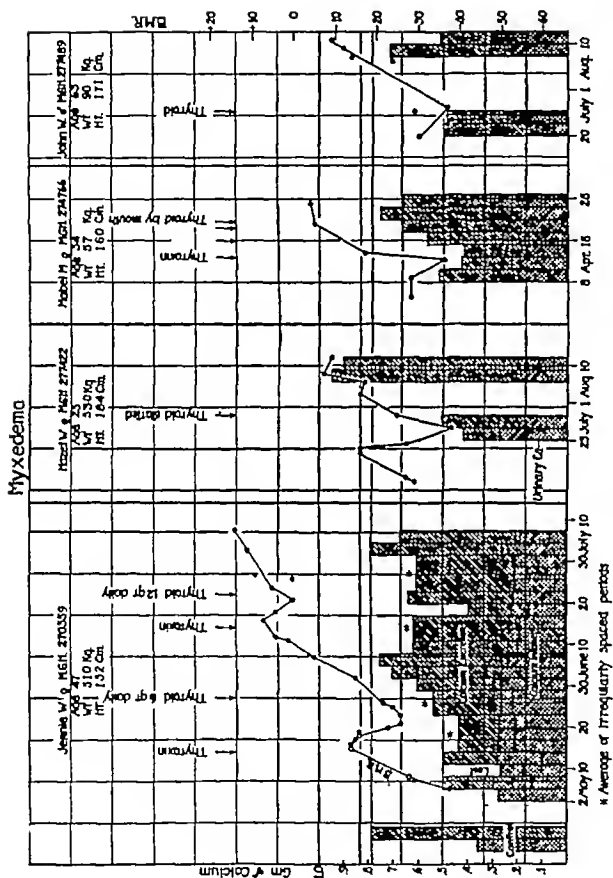


CHART 3 A GRAPHIC REPRESENTATION OF THE CALCIUM METABOLISM DATA GIVEN IN TABLE 6, IN THE CASES OF JENNIE W, HAZEL W, LABEL M, AND JOHN W

Here again the calcium intake, urinary calcium excretion and the fecal calcium excretion are represented as in chart 1

TABLE 2  
Metabolic studies in cases with a high metabolism due to fever and leukemia

| Period number | Phosphorus   |       |       |        |       |       | Calcium |       |       |        |       |       | Nitrogen |       |             |        |      |     | Blood serum |                                     |  |                      | Average (rectal temperature) | Diagnosis |  |
|---------------|--------------|-------|-------|--------|-------|-------|---------|-------|-------|--------|-------|-------|----------|-------|-------------|--------|------|-----|-------------|-------------------------------------|--|----------------------|------------------------------|-----------|--|
|               | Output       |       |       | Intake |       |       | Output  |       |       | Intake |       |       | Output   |       |             | Intake |      |     | Date        | Calcium<br>mgm.<br>per<br>100<br>cc | Phosphorus<br>mgm.<br>per<br>100<br>cc | Basal metabolic rate |                              |           |  |
|               | Urine        | Feces | Total | Urine  | Feces | Total | Urine   | Feces | Total | Urine  | Feces | Total | Urine    | Feces | Total       |        |      |     |             |                                     |  |                      |                              |           |  |
| Christian L.  | I            | 1 307 | 1 070 | 2 377  | 1 759 | 0 348 | 0 492   | 0 840 | 0 323 | 36 32  | 2 7   | 39 02 | 26 55    | 5 353 | October 25  | 8 2    | 3 1  | +21 | 103         | Lymphoblastoma                      |  |                      |                              |           |  |
|               | II           | 1 392 | 1 099 | 2 491  | 2 146 | 0 386 | 0 420   | 0 806 | 0 350 | 30 23  | 4 0   | 34 23 | 28 95    | 5 910 | October 29  | 8 02   | 3 01 |     |             | Tertiary syphilis                   |  |                      |                              |           |  |
|               | III          | 1 289 | 1 013 | 2 302  | 2 342 | 0 343 | 0 649   | 0 992 | 0 382 | 58 49  | 4 2   | 62 69 | 31 87    | 302   | November 5  | 8 9    | 3 01 |     | 102         |                                     |  |                      |                              |           |  |
|               | Average      |       |       |        |       |       | 0 359   | 0 520 | 0 879 | 0 351  |       |       |          |       | November 12 | 8 2    | 5 3  |     | 102         |                                     |  |                      |                              |           |  |
| Myron G B     | I            | 2 795 | 1 100 | 3 895  | 2 460 | 1 105 | 0 893   | 1 998 | 0 356 | 34 52  | 3 53  | 38 05 | 35 3     | 6 633 | March 14    |        |      | +37 | 98          | Myelogenous leukemia                |  |                      |                              |           |  |
|               | II           | 2 448 | 1 143 | 3 591  | 2 478 | 1 053 | 0 673   | 1 726 | 0 366 | 67 00  | 3 55  | 70 55 | 35 5     | 6 693 | March 16    |        |      | +27 | 99          |                                     |  |                      |                              |           |  |
|               | Average      |       |       |        |       |       | 1 079   | 0 783 | 1 862 | 0 361  |       |       |          |       |             |        |      |     |             |                                     |  |                      |                              |           |  |
|               |              |       |       |        |       |       |         |       |       |        |       |       |          |       |             |        |      |     |             |                                     |  |                      |                              |           |  |
| Charles A.    | I            | 1 196 | 0 635 | 1 831  | 1 301 | 0 239 | 0 510   | 0 749 | 0 223 | 29 96  | 2 33  | 32 29 | 23 35    | 356   | April 18    | 8 2    | 4 0  | +19 | 102         | Subacute bacterial endocarditis     |  |                      |                              |           |  |
|               | II           | 0 675 | 0 513 | 1 188  | 1 134 | 0 317 | 0 368   | 0 685 | 0 239 | 26 8   | 2 43  | 29 23 | 24 34    | 864   | April 21    |        |      | +33 | 102         | Streptococcus viridans              |  |                      |                              |           |  |
|               | Average      |       |       |        |       |       | 0 278   | 0 439 | 0 717 | 0 231  |       |       |          |       | April 25    |        |      |     |             |                                     |  |                      |                              |           |  |
|               |              |       |       |        |       |       |         |       |       |        |       |       |          |       |             |        |      |     |             |                                     |  |                      |                              |           |  |
| Jennie R      | I            | 1 520 | 0 430 | 1 950  | 1 240 | 0 305 | 0 248   | 0 553 | 0 161 | 23 85  | 2 16  | 26 01 | 21 62    | 831   |             |        |      | +41 | 99          | Myelogenous leukemia                |  |                      |                              |           |  |
|               | 2 day period |       |       |        |       |       |         |       |       |        |       |       |          |       |             |        |      |     |             |                                     |  |                      |                              |           |  |
|               | II           | 1 478 | 0 714 | 2 192  | 2 077 | 0 271 | 0 498   | 0 769 | 0 228 | 27 20  | 2 96  | 30 16 | 29 64    | 663   |             |        |      |     |             |                                     |  |                      |                              |           |  |
|               | III          | 1 368 | 0 634 | 2 002  | 2 160 | 0 242 | 0 421   | 0 663 | 0 244 | 26 84  | 3 20  | 30 04 | 32 05    | 647   |             |        |      |     |             |                                     |  |                      |                              |           |  |
|               | Average      |       |       |        |       |       | 0 272   | 0 389 | 0 661 | 0 211  |       |       |          |       |             |        |      |     |             |                                     |  |                      |                              |           |  |

again that thyroid increases the endogenous calcium metabolism, for the extra elimination must come from the tissues, and presumably mostly from the bones. Similarly, two cases of parathyroid tetany with very low calcium levels in both their blood and excreta responded to thyroid therapy by a distinct elevation not only of their calcium excretion but also of their serum calcium level. This effect was prolonged and greatly benefited their general condition (12).

It is thus definitely established that increased thyroid secretion augments the excretion of calcium. This effect of thyroid secretion on calcium excretion is greater than its effect upon total metabolism. Thus the percentage variations from the normal controls, as shown in chart 4, indicate this marked difference, although the limits of variation of calcium metabolism are not as definitely established as those for the basal metabolic rate. The differences, however, exceed greatly the limits of variation in our normal series.

The marked effect of thyroid activity in three women can well be compared (Elizabeth B. and Christine B., who had Graves' disease, and Hazel W. who had myxedema). They were all young, and Elizabeth B. and Hazel W. were remarkably similar in age, height and weight. The thyroid effects are obvious from table 1.

5 *In other states of increased basal metabolism.* Is the high calcium excretion of hyperthyroidism dependent on the increased thyroid secretion, or is it merely an accompaniment of an increased metabolism? This question could only be answered by determining the calcium excretion in cases with a high metabolism due to factors other than the thyroid, such as prolonged fever and leukemia. Three such cases—two suffering from subacute bacterial endocarditis, and one from lymphatic leukemia—showed a normal calcium excretion on our routine diet. A fourth case (M. G. B.), suffering from myelogenous leukemia, showed an elevated calcium excretion approaching that found in hyperthyroidism. Three of the four cases, therefore, demonstrated normal calcium excretion in spite of an elevation of general metabolism. The basal metabolic rates in these four cases varied from plus 21 to plus 41 per cent. We may conclude therefore that an increased calcium excretion is not necessarily a function of an elevated metabolic rate alone.

*X-rays of bones in exophthalmic goiter*

It is not to be expected that all cases of hyperthyroidism would show osteoporosis of the bones because of this increased calcium elimina-



FIG 1 SINGLE X-RAY OF THE HANDS OF THREE WOMEN OF SIMILAR AGE, SIZE AND WEIGHT

*C* is a normal control, *E* is the hand of a patient who is known to have had exophthalmic goiter for seventeen years, *M* is the hand of a case of severe myxedema

tion, which could probably be compensated for by a diet containing an adequate amount of calcium. In some cases of long duration, however, this increased calcium loss from the bones may be apparent in x-ray pictures. This is obvious in the x-ray pictured in figure 1, where

the hands of a patient with exophthalmic goiter of 17 years' duration, a case of myxedema, and a normal control are all shown in the same exposure. These individuals (all women) were approximately the same as regards age, weight and the shape of the hands. The marked

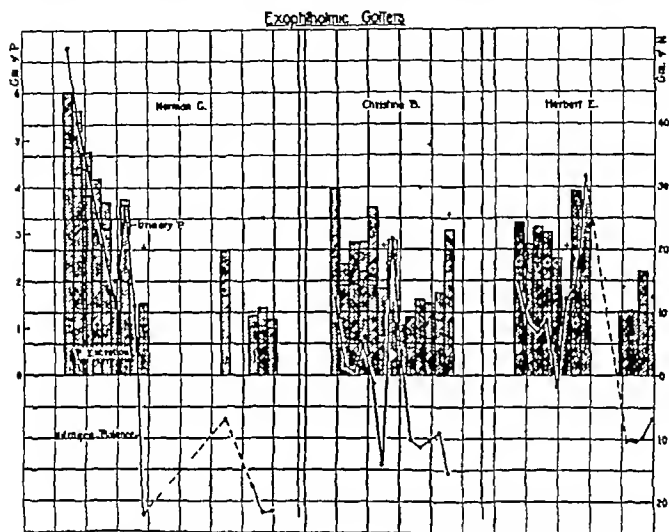


CHART 5 A GRAPHIC REPRESENTATION OF THE PHOSPHORUS METABOLISM DATA GIVEN IN TABLE 4 IN 3 CASES OF EXOPHTHALMIC GOITER

The phosphorus intake is represented by a cross, the heavy black line divides the fecal and urinary phosphorus excretions, the part of the column below being the urinary phosphorus excretion, and the part above being the fecal phosphorus excretion. The nitrogen balance is represented by  $\circ$ — $\circ$ , that above the zero line represents a negative nitrogen balance, and that below, a positive nitrogen balance.

loss of calcium in this case of hyperthyroidism is very striking. We have seen one similar case, although less marked. This observation of osteoporosis in thyroid disease has recently been confirmed by Plummer (13).

*Phosphorus metabolism*

Phosphorus metabolism determinations were made in connection with those of calcium in all of these experiments. This gave us an opportunity to find out whether the calcium loss from the body as a result of thyrotoxicosis represents calcium phosphate withdrawn from the bones. If that were the case there should be a ratio such that the calcium loss to the phosphorus loss would equal that of calcium to phosphorus in tertiary calcium phosphate ( $\text{Ca/P} = 1.93$ ). In addition a correction should be introduced for the phosphorus liberated

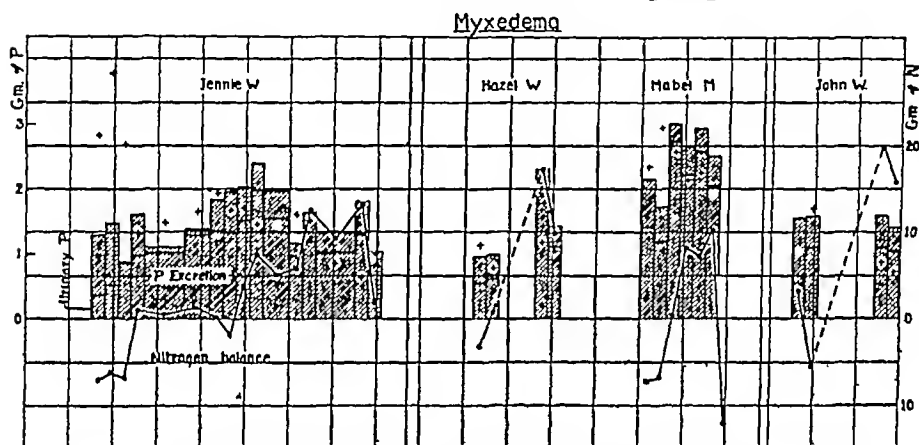


CHART 6 A GRAPHIC REPRESENTATION OF THE PHOSPHORUS METABOLISM DATA GIVEN IN TABLE 6 ON FOUR CASES OF MYXEDEMA

The phosphorus intake, urinary phosphorus excretion and fecal phosphorus excretion are represented as in chart 5

in protein metabolism ( $\text{N/P} = 17.4$ ). In table 3 there is included the actual phosphorus balance and the theoretical phosphorus balance calculated from the calcium and nitrogen balances as above described. In general the actual and theoretical balances agree as closely as could be expected. This supports the supposition that the increased calcium excretion represents, for the most part at any rate, calcium phosphate withdrawn from the bones. That some of this calcium may represent calcium carbonate withdrawn from the bones as suggested by Goto (14) is of course possible.

TABLE 3  
*Phosphorus excretion*

|   | Period number | Phosphorus excretion |            | Phosphorus calculated from |                     | Basal metabolic rate | Weight | Remarks                                 |
|---|---------------|----------------------|------------|----------------------------|---------------------|----------------------|--------|---|
|   |               | Actual               | Calculated | Endogenous calcium         | Endogenous nitrogen |                      |        |   |
| Exophthalmic goiter                     |               |                      |            |                            |                     |                      |        |   |
| Norman, G<br>Age 29<br>(see Table 4)    |               | GRAMS                | GRAMS      | GRAMS                      | GRAMS               | PER CENT             | KGS.   |   |
|   | II            | -4 99                | -5 37      | -2 37                      | -3 00               | +86                  | 56 3   |   |
|   | III           | -4 52                | -4 39      | -2 08                      | -2 31               | +76                  |        | Lugol's solution started                |
|   | IV            | -3 92                | -3 81      | -1 88                      | -1 93               | +79                  |        |   |
|   | V             | -3 10                | -3 01      | -1 52                      | -1 49               | +52                  | 48 3   |   |
|   | VI            | -2 50                | -2 40      | -1 34                      | -1 06               | +56                  | 48 3   |   |
|   | VII           | -0 60                | -1 88      | -1 24                      | -0 64               | +41                  | 48 2   | Subtotal thyroidectomy                  |
|   | VIII          | -3 55                | -2 72      | -1 19                      | -1 53               |                      |        |   |
|   | IX            | -0 06                | -1 21      | -0 58                      | -0 63               | +44                  |        | Lugol's solution discontinued           |
|   | X             | +1 21                | +0 19      | -1 11                      | +1 30               | +19                  | 46 0   | House diet for 3 weeks                  |
|   | XI            |                      |            |                            |                     |                      |        |   |
|   | XII           | +0 285               | -1 03      | -1 43                      | +0 40               | +26                  | 48 0   | Tonsillectomy<br>House diet, for 1 week |
|   | XIII          | +1 667               | -0 35      | -1 02                      | +0 67               |                      |        |   |
|   | XIV           | +1 925               |            |                            | +1 27               | +18                  |        |   |
|   | XV            | +2 180               | +0 57      | -0 68                      | +1 25               |                      | 48 3   |   |
| Elizabeth, B<br>Age 27<br>(see Table 4) | II            | -0 61                | -1 20      | -0 73                      | -0 47               | +70                  | 49 0   |   |
|   | III           | -2 59                | -2 40      | -0 65                      | -1 75               |                      |        | Lugol's solution started                |
|   | IV            | -1 15                | -1 28      | -0 40                      | -0 88               | +40                  | 46 7   |   |
|   | V             | -1 13                | -0 59      | -0 59                      | 0                   | +43                  | 45 5   |   |
|   | VI            | -0 95                | -0 94      | -0 77                      | -0 17               |                      |        |   |
|   | VII           | -0 71                | -0 88      | -0 57                      | -0 31               | +16                  | 46 5   |   |
|   | VIII          | -0 06                | +0 07      | -0 33                      | +0 40               |                      |        | Subtotal thyroidectomy                  |
|   | IX            | +0 16                | +0 30      | -0 30                      | +0 60               |                      |        |   |
|   | X             | -1 16                | -0 71      | -0 29                      | -0 42               |                      |        |   |
|   | XI            | +0 01                | -0 36      | -0 30                      | -0 06               |                      |        |   |
|   | XII           | +1 06                | +0 22      | -0 32                      | +0 54               | +7                   | 43 9   |   |
|   | XIII          | +1 32                | +0 67      | -0 12                      | +0 79               |                      |        |   |
|   | XIV           | +0 69                | +0 15      | -0 43                      | +0 58               |                      |        |   |
|   | XV            | +0 45                | +0 39      | -0 42                      | +0 81               | +8                   | 53 4   |   |

TABLE 3—Continued

|  | Period number | Phosphorus excretion |            | Phosphorus calculated from |                     | Basal metabolic rate | Weight | Remarks               |
|--|---------------|----------------------|------------|----------------------------|---------------------|----------------------|--------|-----------------------|
|  |               | Actual               | Calculated | Endogenous calcium         | Endogenous nitrogen |                      |        |                       |
| Normal controls                        |               |                      |            |                            |                     |                      |        |                       |
| Clark H<br>Age 25<br>(see Table 7)     |               | grams                | grams      | grams                      | grams               | per cent             | kgs    |                       |
|  | I             | -1 69                | -1 08      | -0 30                      | -0 78               | 0                    |        |                       |
|  | II            | -0 04                | -0 36      | -0 27                      | -0 09               |                      |        |                       |
|  | III           | -1 13                | -0 80      | -0 34                      | -0 46               | -6                   |        | Thyroid started       |
|  | IV            | -1 83                | -0 89      | -0 27                      | -0 62               |                      |        |                       |
|  | V             | -1 08                | -2 00      | -0 54                      | -1 46               | +16                  |        | Thyroid stopped       |
|  | VI            | -0 85                | -1 19      | -0 51                      | -0 68               |                      |        |                       |
| Clement I K<br>Age 27<br>(see Table 7) | VII           | -4 03                | -1 46      | -0 55                      | -0 91               | +48                  |        |                       |
|  | I             | -0 11                | -0 43      | -0 12                      | -0 31               | -15                  |        |                       |
|  | II            | -0 57                | -0 60      | -0 27                      | -0 33               | -12                  |        |                       |
|  | III           | -0 85                | -0 62      | -0 22                      | -0 40               | -7                   |        | Thyroid started       |
|  | IV            | -1 13                | -0 66      | -0 18                      | -0 48               |                      |        |                       |
|  | V             | -0 80                | -0 81      | -0 33                      | -0 48               | +8                   |        | Thyroid stopped       |
|  | VI            | -1 34                | -1 88      | -0 36                      | -1 52               |                      |        |                       |
| VII                                    | -0 68         | -0 94                | -0 29      | -0 65                      | +2                  |                      |        |                       |
| Myxedema                               |               |                      |            |                            |                     |                      |        |                       |
| Hazel B W<br>Age 23<br>(see Table 6)   | I             | +0 18                | +0 09      | -0 10                      | +0 19               | -38                  | 52 3   |                       |
|  | II            | +0 19                | -0 20      | -0 17                      | -0 03               | -25                  |        | Thyroid, grs VI daily |
|  | III           | -0 75                | -0 52      | -0 30                      | -0 22               | -16                  | 50 6   |                       |
|  | IV            | -1 12                | -1 36      | -0 36                      | -1 00               | -7                   |        |                       |
|  | V             | -0 40                | -0 93      | -0 35                      | -0 58               | -9                   | 50 7   |                       |

## DISCUSSION

Thyroid secretion has been shown to increase markedly calcium excretion. The cause of this is as yet undetermined. There are several possibilities.

1 *A stimulating effect on the parathyroid glands directly or through their sympathetic innervation.* The absence of any marked rise in the serum calcium is very much against this, although Hunter and Aub (15) reported a case in which the administration of Collip's parathyroid extract increased the calcium excretion without appreciably rais-

ing the serum calcium level. Work now in progress will probably determine this possible relationship.

2 *A method of neutralizing the acid products of an increased metabolism.* The neutralization of the phosphorus set free as a result of the increased protein metabolism might account for the increased calcium excretion. Such an explanation would be analogous to the increased calcium output following the injection of phosphates found by Greenwald (16). However, unpublished observations (14) from this laboratory, on normal people following the administration of large amounts of acid phosphate, showed that a large part of this neutralization was accomplished by ammonia. The increased calcium excretion in these cases was not as marked as was observed in our cases of exophthalmic goiter. The other acid products resulting from an increased protein metabolism might call upon the calcium deposits in a similar manner. Studies on the total acid base metabolism in hyperthyroidism and myxedema are now being made and should help decide this point.

3 *The remaining explanation, namely a direct stimulating catabolic effect on the calcium deposits in the bones,* seems the most likely one, and this would be in agreement with the general action of thyroid on other body tissues. It remains, however, for future work to establish this.

#### CONCLUSIONS

1 The calcium excretion in patients with exophthalmic goiter and in those with hyperfunctioning thyroid adenomata is increased markedly above the normal. This increase (231 per cent) is far greater than the increase in basal metabolic rate (55 per cent).

2 The ingestion of thyroid by normal individuals likewise increases the calcium elimination.

3 The calcium excretion in myxedema is markedly diminished below that found in normal individuals.

4 A marked increase in phosphorus excretion was also found. This increase was quantitatively such as to suggest that most of the calcium excreted came from tertiary calcium phosphate in the bones.

5 This high rate of calcium elimination is not obvious in the blood,

TABLE 4  
*Exophthalmic goiter*

| Dates of period | Period number | Phosphorus |       |        |        | Calcium |        |        |       | Nitrogen |      |         |            | Total caloric intake | Blood serum |          |      | Basal metabolic rate |       | Weight |      | Treatment and remarks  |
|-----------------|---------------|------------|-------|--------|--------|---------|--------|--------|-------|----------|------|---------|------------|----------------------|-------------|----------|------|----------------------|-------|--------|------|--|
|                 |               | Output     |       | Intake | Output |         | Intake | Output |       | Intake   | Date | Calcium | Phosphorus |                      | Date        | Per cent | Date | kgm                  |       |        |      |  |
|                 |               | Urine      | Feces |        | Urine  | Feces   |        | Urine  | Feces |          |      |         |            |                      |             |          |      |                      | Urine | Feces  |      |  |
| IV/10           | I             | 2.28       | 0.96  | 3.24   | 2.40   | 0.53    | 0.99   | 1.52   | 0.32  | 33.9     | 7.4  | 41.3    | 26.5       | 8.215                | IV/10       | 11.5     | 2.8  | IV/7                 | +48   | IV/10  | 56.2 | IV/20 Lugol's M v t i d<br><br>IV/29, lobar pneum until V/9<br><br>V/16, Lugol's M v t i d |
| IV/13           | II            | 2.06       | 0.74  | 2.80   | 2.02   | 0.73    | 0.66   | 1.39   | 0.32  | 29.8     | 8.5  | 38.3    | 29.9       | 8.516                | IV/10       | 11.5     | 2.8  | IV/10                | +42   | IV/10  | 56.2 |  |
| IV/16           | III           | 2.36       | 0.79  | 3.15   | 2.32   | 0.79    | 0.91   | 1.70   | 0.33  | 38.8     | 3.6  | 42.4    | 35.5       | 8.222                | IV/17       | 9.6      | 2.8  | IV/17                | +47   | IV/17  | 55.0 |  |
| IV/18           | IV            | 2.02       | 0.99  | 3.01   | 2.37   | 0.83    | 0.87   | 1.70   | 0.35  | 35.3     | 7.1  | 42.4    | 33.7       | 8.652                | IV/17       | 9.6      | 2.8  | IV/17                | +47   | IV/17  | 55.0 |  |
| IV/22           | V             | 1.73       | 0.76  | 2.49   | 2.37   | 0.83    | 0.87   | 1.70   | 0.35  | 35.3     | 7.1  | 42.4    | 33.7       | 8.652                | IV/22       | 9.6      | 2.8  | IV/23                | +19   | IV/22  | 54.8 |  |
| IV/26           | VI            | 1.66       |       | 2.75   | 0.43   |         |        |        | 0.38  | 19.5     | 3.5  | 23.0    | 35.3       | 9.632                | IV/22       | 9.6      | 2.8  | IV/23                | +19   | IV/22  | 54.8 |  |
| IV/28           | VII           | 2.74       | 1.18  | 3.92   | 1.11   | 0.65    | 0.99   | 1.64   | 0.38  | 19.5     | 3.5  | 23.0    | 35.3       | 9.632                | IV/22       | 9.6      | 2.8  | IV/23                | +19   | IV/22  | 54.8 |  |
| V/1             | VIII          | 3.03       | 0.61  | 3.64   | 0.47   | 0.09    | 0.35   | 0.44   | 0.21  | 26.8     | 10.1 | 36.9    | 12.6       | 3.471                | IV/30       | 10.5     | 3.4  | IV/28                |       | IV/28  | 55.4 |  |
| V/11            | IX            | 0.75       | 0.62  | 1.37   | 1.89   | 0.21    | 0.70   | 0.91   | 0.30  | 12.3     | 3.9  | 16.2    | 30.7       | 5.538                | IV/30       | 10.5     | 3.4  | IV/28                |       | IV/28  | 55.4 |  |
| V/16*           | X             | 1.51       | 0.70  | 2.21   | 2.43   | 0.26    | 0.52   | 0.78   | 0.36  | 18.5     | 4.7  | 23.2    | 33.1       | 8.929                | V/14        | 8.9      | 2.5  | V/15                 | +14   | V/17   | 52.0 |  |
| V/18*           | XI            | 1.04       | 0.29  | 1.33   | 1.66   | 0.14    | 0.39   | 0.53   | 0.24  | 12.4     | 3.5  | 15.9    | 22.7       | 6.220                | V/14        | 8.9      | 2.5  | V/15                 | +14   | V/17   | 52.0 |  |
| V/21            | XII           |            |       |        |        |         |        |        |       |          |      |         |            |                      | V/21        | 9.7      | 3.3  | V/20                 | +18   | V/21   | 53.6 |  |
| V/23            |               |            |       |        |        |         |        |        |       |          |      |         |            |                      | V/21        | 9.7      | 3.3  | V/20                 | +18   | V/21   | 53.6 |  |
|                 |               |            |       |        |        |         |        |        |       |          |      |         |            |                      | VI/12       |          |      | VI/12                | +5    |        |      |  |

Herbert I. Age 34



TABLE 4—Continued

| Dates of period | Period number | Phosphorus |       |        | Calcium |       |        | Nitrogen |       |        | Total caloric intake | Blood serum     |                 |            | Basal metabolic rate |         | Weight |        | Treatment and remarks |  |                       |
|-----------------|---------------|------------|-------|--------|---------|-------|--------|----------|-------|--------|----------------------|-----------------|-----------------|------------|----------------------|---------|--------|--------|-----------------------|--|-----------------------|
|                 |               | Output     |       | Intake | Output  |       | Intake | Output   |       | Intake |                      | Date            | Calcium         | Phosphorus | Date                 | Percent | Date   | kgm    |                       |  |                       |
|                 |               | Urine      | Feces |        | Urine   | Feces |        | Urine    | Feces |        |                      |                 |                 |            |                      |         |        |        |                       | Total  |                       |
| II/21*          |               | gm         | gm    | gm     | gm      | gm    | gm     | gm       | gm    | gm     |                      | mgm. per 100 cc | mgm. per 100 cc |            |                      | II/17   | +76    | II/19  | 42 9                  | II/19, transferred to Metabolism Ward complete rest in bed, low calcium diet |                       |
| II/25           | I             | 2 51       | 1 48  | 3 99   | 1 23    | 0 80  | 2 03   | 0 33     | 24 6  | 11 7   | 46 3                 | 34 1            | 7 437           | 10 0       | 2 9                  | II/25   |        | II/19  | 43 5                  | II/28, 2 grams calcium lactate by mouth                                      |                       |
| II/28           | II            | 1 91       | 0 45  | 2 36   | 1 48    | 0 71  | 2 19   | 0 30     | 27 3  | 6 5    | 33 8                 | 32 3            | 7 630           |            |                      | II/24   | +51    | II/24  |                       | III/6 began Lugol's Mx.t.d   |                       |
| III/3           | III           | 1 93       | 0 89  | 2 82   | 1 46    | 0 61  | 2 07   | 0 32     | 28 3  | 5 0    | 33 3                 | 32 5            | 7 816           |            |                      | III/3   | +57    | III/3  | 42 9                  | III/12, subtotal thyroidec tomy  |                       |
| III/6           | IV            | 2 20       | 0 62  | 2 82   | 1 98    | 0 36  | 2 34   | 0 27     | 26 1  | 4 3    | 30 4                 | 25 8            | 7 224           |            |                      | III/3   |        | III/3  | 41 9                  |  |                       |
| III/10†         | V             | 2 09       | 1 50  | 3 59   | 2 25    | 1 47  | 1 09   | 2 56     | 0 35  | 28 5   | 11 2                 | 39 7            | 8 253           | 9 5        | 4 3                  | III/8   |        | III/10 |                       |  |                       |
| III/12††        | VI            | 0 84       | 1 00  | 1 84   | 2 75    | 0 72  | 0 69   | 1 41     | 0 37  | 13 0   | 6 4                  | 19 4            | 9 127           |            |                      | III/15  |        | III/16 |                       |  |                       |
| III/16          | VII           | 2 42       | 0 44  | 2 86   | 0 44    | 0 88  | 0 43   | 1 31     | 0 02  | 19 5   | 3 8                  | 23 3            | 1 8             | 651        | 9 0                  | 2 2     | III/16 | +11    | III/16                | 41 9   |                       |
| III/18          | VIII          | 0 71       | 0 35  | 1 06   | 0 50    | 0 57  | 0 42   | 0 99     | 0 16  | 8 0    | 2 1                  | 10 1            | 6 9             | 3 221      |                      |         | III/16 |        | III/16                | 41 9   |                       |
| III/22          | IX            | 0 59       | 0 62  | 1 21   | 1 92    | 0 31  | 0 54   | 0 85     | 0 27  | 7 2    | 3 3                  | 10 5            | 20 8            | 6 345      |                      |         | III/16 |        | III/16                | 41 9   |                       |
| III/24          | X             | 0 68       | 0 72  | 1 40   | 3 99    | 0 58  | 0 52   | 1 10     | 0 32  | 12 2   | 4 7                  | 16 9            | 28 1            | 9 150      |                      |         | III/16 |        | III/16                | 41 9   |                       |
| III/27          | XI            | 0 79       | 0 74  | 1 53   | 4 88    | 0 42  | 0 59   | 1 01     | 0 31  | 13 1   | 4 8                  | 17 9            | 28 3            | 9 378      |                      |         | III/31 | -7     | III/31                | 43 5   | IV/2, Lugol's stopped |
| III/30          | XII           | 1 19       | 0 56  | 1 75   | 4 88    | 0 40  | 0 48   | 0 88     | 0 31  | 14 1   | 5 1                  | 19 2            | 28 3            | 9 378      | 9 3                  | 2 5     | III/31 |        | III/31                | 43 5   |                       |
| IV/2            | XIII          | 2 21       | 0 88  | 3 09   | 3 42    | 0 34  | 0 66   | 1 00     | 0 32  | 11 9   | 0 9                  | 12 8            | 28 3            | 9 124      | 9 5                  | 2 5     | IV/9   | +19    | IV/9                  | 45 6   |                       |
| IV/3**          | XIV           | 0 68       | 0 70  | 1 38   | 3 97    | 0 19  | 0 41   | 0 60     | 0 29  | 6 6    | 1 8                  | 8 4             | 27 3            | 9 378      |                      |         | IV/20  | -2     | IV/20                 | 46 2   |                       |

\* Period I is 4 days, reduced in the above table to the equivalent of 3 days.

\*\* Period XIV is 14 days, results being multiplied by 2 to give equivalent 3-day amounts.

† Period V Urine About 500 cc. may belong to period VI.

†† Period VI Stool Contains 400 cc of urine.

|       |      |      |      |      |      |      |      |      |      |      |       |        |       |       |      |     |        |     |        |      |       |                      |
|-------|------|------|------|------|------|------|------|------|------|------|-------|--------|-------|-------|------|-----|--------|-----|--------|------|-------|----------------------|
| I/13  | II   | 2 09 | 0 73 | 2 83 | 2 21 | 1 08 | 0 69 | 1 77 | 0 29 | 34 7 | 3 528 | 2 30 1 | 5 686 | I/14  | 9 8  |     | I/14   | +79 | I/14   | 40 6 | I/20  | Lugol's M x t.l.d.   |
| I/18  | III  | 2 88 | 1 01 | 3 99 | 1 31 | 0 70 | 0 84 | 1 54 | 0 23 | 39 0 | 7 847 | 8 16 9 | 2 977 | I/18  | +70  |     | I/18   | +70 | I/18   | 40 0 | I/22  | Lugol's M x t.l.d.   |
| I/21  |      |      |      |      |      |      |      |      |      |      |       |        |       |       |      |     |        |     |        |      | I/24  | Lugol's M x t.l.d.   |
| I/24  | IV   | 1 03 | 0 93 | 1 96 | 0 81 | 0 34 | 0 97 | 1 01 | 0 20 | 21 4 | 6 427 | 8 12 3 | 2 409 | I/24  | 10 2 | 4 3 | I/24   | +40 | I/24   | 46 7 | I/25  | Lugol's discontinued |
| II/1  | V    | 1 68 | 0 93 | 2 61 | 1 48 | 0 77 | 0 68 | 1 45 | 0 27 | 35 7 | 6 372 | 2 22 2 | 8 665 | II/1  | 8 9  | 4 7 | II/1   | +43 | II/1   | 45 5 | II/1  | Lugol's M x t.l.d.   |
| II/4  | VI   | 1 88 | 0 63 | 2 31 | 1 56 | 1 28 | 0 34 | 1 92 | 0 28 | 21 7 | 4 766 | 4 23 5 | 7 048 | II/5  | 8 9  | 4 7 | II/5   | +16 | II/5   | 46 3 | II/11 | Lugol's discontinued |
| II/7  | VII  | 1 28 | 0 82 | 2 10 | 1 39 | 0 81 | 0 59 | 1 40 | 0 26 | 20 1 | 5 425 | 3 20 1 | 6 881 |       |      |     |        |     |        |      | II/13 | operation subtotal   |
| II/10 | VIII | 0 91 | 0 75 | 1 66 | 1 60 | 0 35 | 0 60 | 0 95 | 0 29 | 15 3 | 3 116 | 4 23 3 | 7 395 | II/12 | 8 9  | 2 4 | II/12  |     |        |      | II/15 | operation subtotal   |
| II/13 | IX   | 0 75 | 0 78 | 1 51 | 1 65 | 0 33 | 0 47 | 1 00 | 0 30 | 10 8 | 3 514 | 3 24 8 | 7 485 | II/15 | 8 9  |     | II/15  |     |        |      | II/15 | Lugol's M v t.l.d.   |
| II/16 | X    | 0 97 | 0 32 | 1 20 | 0 14 | 0 37 | 0 29 | 0 66 | 0 08 | 8 0  | 2 510 | 3 3 2  | 872   |       |      |     |        |     |        |      | II/23 | Lugol's M v t.l.d.   |
| II/19 | XI   | 0 51 | 0 24 | 0 73 | 0 76 | 0 29 | 0 62 | 0 91 | 0 31 | 11 0 | 3 914 | 9 13 8 | 4 015 | II/20 | 6 42 |     | II/20  |     |        |      | II/28 | Lugol's discontinued |
| II/23 | XII  | 0 35 | 0 41 | 0 76 | 1 80 | 0 23 | 0 73 | 0 96 | 0 32 | 8 6  | 7 616 | 2 25 6 | 6 853 | II/22 | 9 6  |     | II/22  |     |        |      |       |                      |
| II/25 | XIII | 0 39 | 0 14 | 0 53 | 1 83 | 0 34 | 0 22 | 0 56 | 0 31 | 11 0 | 1 412 | 4 26 1 | 6 869 |       |      |     |        |     |        |      |       |                      |
| II/28 | XIV  | 0 32 | 0 57 | 0 89 | 1 58 | 0 51 | 0 67 | 1 18 | 0 33 | 12 6 | 2 915 | 5 25 5 | 6 819 | III/3 | 10 0 | 3 2 | III/3  |     |        |      |       |                      |
| III/5 | XV   | 0 37 | 0 77 | 1 14 | 1 38 | 0 42 | 0 72 | 1 14 | 0 32 | 7 6  | 3 811 | 4 23 5 | 6 819 |       |      |     | III/31 | +8  | III/31 | 53 4 |       |                      |

|       |      |      |      |      |      |      |      |      |      |      |       |        |       |       |      |     |       |     |       |      |       |                    |
|-------|------|------|------|------|------|------|------|------|------|------|-------|--------|-------|-------|------|-----|-------|-----|-------|------|-------|--------------------|
| IV/16 | I    | 2 16 | 0 74 | 2 90 | 2 16 | 1 04 | 0 56 | 1 60 | 0 28 | 30 1 | 7 537 | 6 32 9 | 7 686 | I/16  | 10 8 | 3 0 | I/16  | +87 | I/16  | 43 1 | IV/23 | Lugol's M x t.l.d. |
| IV/21 | II   | 1 81 | 0 89 | 2 70 | 1 40 | 1 15 | 0 79 | 1 94 | 0 21 | 28 9 | 2 431 | 3 23 0 | 6 367 | IV/22 | 9 9  | 3 9 | IV/22 | +73 | IV/22 | 42 2 |       |                    |
| IV/24 | III  | 1 91 | 0 85 | 2 76 | 1 49 | 1 35 | 0 66 | 2 01 | 0 21 | 27 9 | 3 831 | 7 23 6 | 6 432 | IV/28 | 9 9  | 3 3 | IV/28 | +62 | IV/28 |      |       |                    |
| IV/28 | IV   | 1 50 | 0 77 | 2 27 | 1 65 | 1 11 | 0 71 | 1 82 | 0 24 | 17 6 | 4 021 | 6 25 7 | 6 387 | I/30  | 9 9  | 3 3 | I/30  | +57 | I/30  |      |       |                    |
| V/1   | V    | 1 68 | 0 86 | 2 54 | 1 65 | 1 44 | 0 78 | 2 22 | 0 24 | 20 1 | 5 225 | 3 25 6 | 5 798 |       |      |     | V/4   |     | V/4   |      |       |                    |
| V/4   |      |      |      |      |      |      |      |      |      |      |       |        |       |       |      |     |       |     |       |      |       |                    |
| V/6   | VI   | 1 86 | 0 54 | 2 40 | 1 62 | 1 07 | 0 51 | 2 48 | 0 22 | 23 5 | 5 228 | 7 34 1 | 5 931 |       |      |     | V/6   |     | V/6   | 41 8 | V/5   | Lugol's M x t.l.d. |
| V/12  |      |      |      |      |      |      |      |      |      |      |       |        |       |       |      |     |       |     |       |      |       |                    |
| V/15  | VII  | 0 78 | 1 01 | 1 79 | 1 58 | 0 54 | 0 98 | 1 52 | 0 22 | 9 0  | 2 811 | 8 22 7 | 5 682 | V/14  | 9 9  | 3 0 | V/14  | +27 | V/14  | 40 2 | V/6   | operation          |
| V/18  | VIII | 0 72 | 0 76 | 1 48 | 1 78 | 0 61 | 0 59 | 1 20 | 0 25 | 12 8 | 4 517 | 1 24 8 | 6 499 |       |      |     |       |     |       |      |       |                    |
| V/21  | IX   | 0 25 | 0 78 | 1 03 | 1 97 | 0 56 | 0 53 | 1 09 | 0 25 | 16 4 | 3 019 | 4 28 6 | 6 991 | V/21  | 9 0  | 5 7 | V/21  |     | V/21  | 42 6 | V/9   | Lugol's stopped    |
| V/25  | X    | 0 50 | 0 66 | 1 10 | 1 45 | 0 56 | 0 41 | 1 00 | 0 15 | 7 4  | 5 012 | 4 17 5 | 4 236 |       |      |     |       |     |       |      |       |                    |

TABLE 4—*Concluded*

| Dates of period   | Period number | Phosphorus |       |       | Calcium |       |       | Nitrogen |       |       | Total caloric intake | Blood serum |         |            | Basal metabolic rate |         | Weight |        | Treatment and remarks |        |      |  |
|-------------------|---------------|------------|-------|-------|---------|-------|-------|----------|-------|-------|----------------------|-------------|---------|------------|----------------------|---------|--------|--------|-----------------------|--------|------|--|
|                   |               | Output     |       |       | Output  |       |       | Output   |       |       |                      | Date        | Calcium | Phosphorus | Date                 | Percent | Date   | Kgm    |                       |        |      |  |
|                   |               | Urine      | Feces | Total | Urine   | Feces | Total | Urine    | Feces | Total |                      |             |         |            |                      |         |        |        |                       |        |      |  |
| George R A Age 24 |               |            |       |       |         |       |       |          |       |       |                      |             |         |            |                      |         |        |        |                       |        |      |  |
|                   | I             | 2 00       | 0 47  | 2 47  | 2 05    | 0 54  | 0 38  | 0 92     | 0 32  | 29 5  | 2 63                 | 1 27        | 8       |            |                      |         |        | III/12 | +58                   | III/12 | 56 5 | III/30 transferred to Metabolism Ward, rest in bed low calcium diet                                  |
| IV/3              | II            | 1 92       | 0 92  | 2 84  | 2 05    | 0 72  | 0 62  | 1 34     | 0 32  | 28 2  | 7 03                 | 2 27        | 8       |            |                      |         |        | III/29 | +49                   | III/29 | 55 4 |  |
| IV/6              | III           | 1 76       | 0 67  | 2 43  | 2 05    | 0 68  | 0 50  | 1 18     | 0 32  | 28 5  | 3 33                 | 1 8         | 29 6    |            |                      |         |        | IV/5   | +31                   | IV/5   | 54 1 |  |
| IV/9              |               |            |       |       |         |       |       |          |       |       |                      |             |         |            |                      |         |        | IV/7   | +23                   | IV/7   | 53 9 | IV/10, Lugol's begun<br>IV/15, transferred to Ward D<br>IV/17, subtotal thyroidectomy, good recovery |
|                   | IV            | 1 81       | 1 04  | 2 85  | 1 91    | 0 67  | 1 02  | 1 69     | 0 36  | 28 6  | 6 43                 | 0 33        | 2       | IV/5       | 10 5                 |         |        | IV/10  | +15                   | IV/10  | 53 8 |  |
| IV/13             | V             | 1 20       | 0 88  | 2 08  | 2 44    | 0 51  | 0 70  | 1 21     | 0 37  | 13 4  | 4 21                 | 6 33        | 7       | IV/12      | 10 6                 |         |        |        |                       |        |      |  |
| (IV/16)*          |               |            |       |       |         |       |       |          |       |       |                      |             |         |            |                      |         |        | IV/23  | +3                    | IV/23  | 52 9 |  |

\* Period V is 2-day period multiplied by 2

\* Period V, a 2-day period multiplied by 3/2

TABLE 5

## Hyperfunctioning adenomas of the thyroid

| Dates of period             | Period number | Phosphorus |       |       | Calcium |       |       | Nitrogen |       |       | Total caloric intake | Blood serum |         | Basal meta-bolic rate |        | Weight | Treatment and remarks |          |     |  |
|-----------------------------|---------------|------------|-------|-------|---------|-------|-------|----------|-------|-------|----------------------|-------------|---------|-----------------------|--------|--------|-----------------------|----------|-----|--|
|                             |               | Output     |       |       | Output  |       |       | Output   |       |       |                      | Date        | Calcium | Phosphorus            | Date   |        |                       | Per cent |     |  |
|                             |               | Urine      | Feces | Total | Urine   | Feces | Total | Urine    | Feces | Total |                      |             |         |                       |        |        |                       |          |     |  |
| Margaret Catherine D Age 47 |               |            |       |       |         |       |       |          |       |       |                      |             |         |                       |        |        |                       |          |     |  |
| IV/16                       | I             | 1 67       | 1 102 | 77 2  | 48 0    | 22 0  | 70    | 0 92     | 0 33  | 24 6  | 5 8                  | 30 4        | 35 57   | 531                   | IV/16  | 10 9   | 4 5                   | IV/5     | +59 | IV/23 operation—lobectomy  |
| IV/19                       | II            | 2 03       | 1 26  | 13 2  | 64 0    | 39 0  | 61    | 1 00     | 0 34  | 29 9  | 6 4                  | 36 3        | 39 78   | 491                   | IV/15  | +31    | 62 6                  | IV/16    | +51 |  |
| IV/23                       | III           | 1 82       | 0 89  | 21 5  | 58 0    | 32 0  | 42    | 0 94     | 0 34  | 31 4  | 6 3                  | 37 7        | 40 19   | 310                   | IV/22  | +29    | 61 8                  | IV/23    | +29 |  |
| IV/25                       | IV            | 1 69       | 1 03  | 24 4  | 60 0    | 63 0  | 52    | 1 15     | 0 32  | 26 6  | 7 1                  | 33 7        | 39 09   | 306                   | IV/28  | +27    | 62 0                  | IV/28    | +27 |  |
| IV/28                       | V             | 2 28       | 0 41  | 22 0  | 13 0    | 26 0  | 25    | 0 31     | 0 81  | 25 4  | 4 4                  | 29 8        | 0 9     | 714                   | IV/30  | 10 1   | 3 2                   | V/3      | +13 |  |
| V/1                         | VI            | 1 14       | 0 60  | 1 74  | 0 90    | 27 0  | 42    | 0 69     | 0 20  | 22 9  | 2 9                  | 22 1        | 13 23   | 918                   | V/7    | 9 6    | 2 5                   | V/10     | +3  |  |
| V/4                         | VII           | 0 71       | 1 00  | 1 71  | 0 37    | 0 80  | 51    | 0 71     | 0 23  | 17 7  | 4 3                  | 22 0        | 29 36   | 460                   | V/25   | +18    | 62 4                  | V/12     | +18 |  |
| V/7                         | VIII          | 0 46       | 0 85  | 1 34  | 2 96    | 21 0  | 43    | 0 64     | 0 30  | 13 0  | 5 5                  | 18 5        | 29 76   | 535                   |        |        |                       |          |     |  |
| V/10                        | IX            | 0 73       | 0 71  | 1 44  | 13 0    | 24 0  | 34    | 0 38     | 0 35  | 14 7  | 4 5                  | 19 2        | 30 26   | 537                   |        |        |                       |          |     |  |
| V/13                        |               |            |       |       |         |       |       |          |       |       |                      |             |         |                       |        |        |                       |          |     |  |
| Sophie Joanne K. Age 58     |               |            |       |       |         |       |       |          |       |       |                      |             |         |                       |        |        |                       |          |     |  |
| VII/20                      | I             | 2 08       | 0 74  | 2 82  | 1 32    | 0 81  | 0 45  | 1 26     | 0 21  | 29 8  | 4 8                  | 34 6        | 20 83   | 977                   | VII/19 | 10 3   | 4 1                   | VII/3    | +67 | Low calcium diet<br>Lugol's M xv t.i.d.<br>VII/31 operation—lobectomy<br>VIII/3 Lugol's discontinued |
| VII/23                      | II            | 1 92       | 0 75  | 2 67  | 1 61    | 0 96  | 0 42  | 1 38     | 0 27  | 26 8  | 5 7                  | 32 5        | 29 54   | 823                   | VII/26 | 9 9    | 5 0                   | VII/22   | +41 |  |
| VII/26                      | III           | 1 72       | 0 60  | 2 32  | 1 49    | 0 97  | 0 48  | 1 45     | 0 68  | 31 5  | 5 9                  | 35 4        | 20 83   | 806                   | VII/27 | 10 0   |                       | VII/26   | +42 |  |
| VII/29                      | IV            | 0 89       | 1 00  | 1 89  | 1 39    | 0 27  | 0 65  | 0 92     | 0 18  | 12 9  | 2 2                  | 15 1        | 15 63   | 306                   |        |        |                       | VIII/5   | +15 |  |
| VIII/5                      | V             | 0 81       | 0 93  | 1 74  | 0 90    | 0 13  | 0 35  | 0 48     | 0 23  | 10 7  | 7 6                  | 18 3        | 16 63   | 430                   |        |        |                       | VIII/10  | -16 |  |
| VIII/11                     | VI            | 0 49       | 0 57  | 1 06  | 1 21    | 0 15  | 0 37  | 0 52     | 0 29  | 9 7   | 7 6                  | 17 3        | 21 74   | 668                   |        |        |                       | IX/13    | +4  |  |
| VIII/15                     |               |            |       |       |         |       |       |          |       |       |                      |             |         |                       |        |        |                       |          |     |  |

Two-day period. Results multiplied by 3/2.

TABLE 6

## Cases of myxedema

| Dates of period  | Period number | Phosphorus      |       |        | Calcium |       |        | Nitrogen |       |        | Total caloric intake | Blood serum |         |            | Basal metabolic rate |          | Weight |        | Treatment and remarks |        |      |  |      |      |
|------------------|---------------|-----------------|-------|--------|---------|-------|--------|----------|-------|--------|----------------------|-------------|---------|------------|----------------------|----------|--------|--------|-----------------------|--------|------|--|------|------|
|                  |               | Output          |       | Intake | Output  |       | Intake | Output   |       | Intake |                      | Date        | Calcium | Phosphorus | Date                 | Per cent | Date   | Kgms   |                       |        |      |  |      |      |
|                  |               | Urine           | Feces |        | Urine   | Feces |        | Urine    | Feces |        |                      |             |         |            |                      |          |        |        |                       |        |      |  |      |      |
|                  |               | Susan F. Age 48 |       |        |         |       |        |          |       |        |                      |             |         |            |                      |          |        |        |                       |        |      |  |      |      |
| VI/3             | I             | 0.80            | 0.88  | 1.68   | 0.52    | 0.07  | 0.61   | 0.68     | 0.14  | 9.3    | 8.1                  | 17.4        | 7.6     | 1.921      | VI/6                 | 9.3      | 3.7    | VI/19  | -33                   | VI/2   | 61.4 | VI/7, thyroxin 10 mgm.<br>VI/10, thyroxin 10 mgm.<br>VI/13, thyroxin 10 mgm. |      |      |
| VI/6             | II            | 0.26            | 0.74  | 1.00   | 0.83    | 0.06  | 0.47   | 0.53     | 0.40  | 6.0    | 6.3                  | 12.3        | 11.6    | 2.306      | VI/6                 | 9.3      | 3.7    | VI/2   | -42                   | VI/7   | 63.0 |  |      |      |
| VI/9             | III           | 1.78            | 0.41  | 2.19   | 1.36    | 0.20  | 0.44   | 0.64     | 1.03  | 16.1   | 1.7                  | 17.8        | 10.8    | 2.248      | VI/12                | 8.8      | 4.4    | VI/5   | -39                   |        |      |  |      |      |
| VI/12            | IV            | 1.39            | 0.70  | 2.09   | 1.62    | 0.14  | 0.57   | 0.71     | 1.75  | 14.0   | 6.1                  | 20.1        | 16.8    | 3.082      | VI/12                | 8.8      | 4.4    | VI/9   | -26                   |        |      |  |      |      |
| VI/15            | V             | 1.17            | 0.80  | 1.97   | 1.00    | 0.10  | 0.67   | 0.77     | 1.85  | 24.2   | 4.5                  | 28.7        | 12.6    | 3.611      |                      |          |        | VI/17  | +1                    | VI/16  | 58.5 |  |      |      |
| VI/18            | VI            | 1.34            | 0.65  | 2.09   | 1.56    | 0.12  | 0.66   | 0.78     | 1.28  | 29.8   | 4.8                  | 34.6        | 18.5    | 3.438      | VI/21                | 9.2      | 6.7    | VI/17  |                       | VI/22  | 54.6 |  |      |      |
| VI/21            | VII           | 1.88            | 0.69  | 2.57   | 1.44    | 0.25  | 0.67   | 0.92     | 1.18  | 34.2   | 4.7                  | 38.9        | 11.6    | 2.694      | VI/26                | 10.3     | 8.8    | VI/22  |                       | VI/24  | 52.0 |  |      |      |
| VI/24            |               |                 |       |        |         |       |        |          |       |        |                      |             |         |            | VI/28                | 11.3     | 7.6    | VI/29  |                       | VI/29  | 46.6 |  |      |      |
| VII/11           |               |                 |       |        |         |       |        |          |       |        |                      |             |         |            | VII/14               | 8.3      | 5.4    | VII/7  |                       | VII/13 | 47.0 |  |      |      |
| VII/13           | VIII          | 0.89            | 0.41  | 1.30   | 1.07    | 0.05  | 0.37   | 0.42     | 0.33  | 11.6   |                      |             |         |            |                      |          |        | VII/13 | -5                    |        |      |  |      |      |
| Jennie W. Age 47 |               |                 |       |        |         |       |        |          |       |        |                      |             |         |            |                      |          |        |        |                       |        |      |  |      |      |
| V/2              | I             | 0.36            | 0.92  | 1.28   | 2.82    | 0.20  | 0.82   | 1.02     | 0.27  | 15.2   | 4.7                  | 19.9        | 27.0    | 5.756      | V/3                  | 9.9      |        |        |                       | V/5    | -38  |  | V/6  | 53.0 |
| V/5              | II            | 0.53            | 0.94  | 1.47   | 3.78    | 0.14  | 0.35   | 0.49     | 0.22  | 9.8    | 6.6                  | 16.4        | 22.7    | 4.499      | V/3                  | 9.9      |        |        |                       | V/5    | -38  | V/6  | 53.0 |      |
| V/9              | III           | 0.87            | Lost  | ?      | 2.68    | 0.17  | Lost   | ?        | 0.26  | 14.0   |                      |             | 23.2    | 4.541      | V/10                 | 10.0     | 2.9    |        |                       | V/8    | -28  |  |      |      |
| V/11             | IV            | 0.99            | 0.61  | 1.60   | 1.31    | 0.19  | 0.31   | 0.50     | 0.29  | 13.0   | 4.2                  | 17.2        | 16.2    | 3.765      | V/10                 | 10.0     | 2.9    |        |                       | V/15   | -14  |  |      |      |
| V/14             |               |                 |       |        |         |       |        |          | 0.28  |        |                      |             | 14.9    | 3.353      | V/17                 | 10.1     | 2.8    |        |                       | V/16   | -14  |  |      |      |
| V/18             | V*            | 1.01            | 0.42  | 1.09   | 1.51    | 0.21  | 0.22   | 0.43     | 0.28  | 15.3   | 3.2                  | 18.5        | 20.1    | 4.639      | V/17                 | 10.1     | 2.8    |        |                       | V/17   | -14  |  |      |      |

| V/23  | VI     |      |      |      |      | 0.28 |      |      | 21.9 | 5.275 | V/74 | 10.0 | 3.5   | V/18   | -16 |  |       |                         |
|-------|--------|------|------|------|------|------|------|------|------|-------|------|------|-------|--------|-----|--|-------|-------------------------|
| V/27  | VIII†  | 1.29 | 0.59 | 1.88 | 1.64 | 0.26 | 0.27 | 0.53 | 0.22 | 16.8  | 3.2  | 20.0 |       |        |     |  | V/26  | 52.2                    |
| V/29  | VIII†  | 1.36 | 0.46 | 1.82 | 1.03 | 0.29 | 0.32 | 0.62 | 0.26 | 20.5  | 4.6  | 24.9 | 5.4   | VI/2   | -13 |  | V/31  | 51.2                    |
| VI/1  | IX     | 1.33 | 0.62 | 1.95 | 1.67 | 0.32 | 0.38 | 0.70 | 0.26 | 20.0  | 2.0  | 22.0 | 5.398 | VI/7   | -5  |  | VI/8  | 50.6                    |
| VI/4  | X      | 1.57 | 0.37 | 2.02 | 1.70 | 0.23 | 0.52 | 0.75 | 0.26 | 24.1  | 5.7  | 27.8 | 5.8   | VI/11  | +1  |  |       |                         |
| VI/7  | XI     | 1.97 | 0.50 | 2.47 | 1.46 | 0.19 | 0.42 | 0.61 | 0.23 | 24.2  | 5.2  | 27.4 | 5.5   | VI/12  | +4  |  |       |                         |
| VI/10 | XII    | 1.56 | 0.40 | 1.96 | 1.45 | 0.18 | 0.43 | 0.61 | 0.26 | 22.2  | 3.0  | 25.2 |       | VI/13  | +4  |  |       |                         |
| VI/14 | XIII†  |      |      |      |      |      |      |      |      |       |      |      |       | VI/14  | +0  |  |       | VI/14 thyroxine 10 mgm. |
| VI/16 | XIV†   |      |      |      |      |      |      |      | 0.20 |       |      |      |       | VI/15  | +11 |  |       |                         |
|       |        |      |      |      |      |      |      |      |      |       |      |      |       | VI/16  | +7  |  |       |                         |
|       |        |      |      |      |      |      |      |      |      |       |      |      |       | VI/17  | +3  |  |       |                         |
| VI/19 | XV     | 0.96 | 0.20 | 1.16 | 1.60 | 0.15 | 0.24 | 0.39 | 0.22 | 19.6  | 1.9  | 21.5 | 5.4   | VI/18  | +4  |  | VI/19 | 50.0                    |
| VI/22 | XVI    | 1.19 | 0.43 | 1.62 | 1.51 | 0.17 | 0.47 | 0.64 | 0.22 | 22.0  | 3.5  | 23.5 |       | VI/19  | +0  |  |       |                         |
|       |        |      |      |      |      |      |      |      |      |       |      |      |       | VI/21  | +0  |  |       |                         |
| VI/26 | XVII** | 1.02 | 0.30 | 1.31 | 0.86 | 0.20 | 0.39 | 0.59 | 0.16 | 18.0  | 2.0  | 20.0 |       | VI/23  | +5  |  | VI/22 | 49.8                    |
| VI/29 | XVIII  |      |      |      |      |      |      |      |      |       |      |      |       | VI/25  | +1  |  | VI/29 | 48.2                    |
| VII/1 | XIX    |      |      |      |      |      |      |      | 0.22 |       |      |      |       | VI/26  | +9  |  |       |                         |
| VII/4 | XX     | 1.30 | 0.50 | 1.80 | 0.66 | 0.26 | 0.52 | 0.78 | 0.23 | 19.0  | 3.4  | 22.4 | 5.9   | VII/3  | -10 |  |       |                         |
| VII/7 | XXI    | 0.82 | 0.21 | 1.03 | 1.00 | 0.20 | 0.46 | 0.66 | 0.18 | 13.9  | 2.6  | 16.5 |       | VII/6  | -8  |  |       |                         |
|       |        |      |      |      |      |      |      |      |      |       |      |      |       | VII/8  | -14 |  | VII/7 | 45.6                    |
|       |        |      |      |      |      |      |      |      |      |       |      |      |       | VII/9  | -13 |  |       |                         |
|       |        |      |      |      |      |      |      |      |      |       |      |      |       | VII/10 | -3  |  |       |                         |

Average of 2 uneven periods.

† Average of 2 uneven periods.

\*\* Average of 5 uneven periods.

TABLE 6—Continued

| Dates of period | Period number | Phosphorus |       |       | Calcium |       |       | Nitrogen |       |       | Total caloric intake | Blood serum |         |            | Basal meta bolic rate |          | Weight |                | Treatment and remarks |       |      |  |
|-----------------|---------------|------------|-------|-------|---------|-------|-------|----------|-------|-------|----------------------|-------------|---------|------------|-----------------------|----------|--------|----------------|-----------------------|-------|------|--|
|                 |               | Output     |       |       | Output  |       |       | Output   |       |       |                      | Date        | Calcium | Phosphorus | Date                  | Per cent | Kgms   |                |                       |       |      |  |
|                 |               | Urine      | Feces | Total | Urine   | Feces | Total | Urine    | Feces | Total |                      |             |         |            |                       |          |        |                |                       |       |      |  |
|                 |               |            |       |       |         |       |       |          |       |       |                      |             |         |            |                       |          |        |                |                       |       |      |  |
| Mabel M Age 34  |               |            |       |       |         |       |       |          |       |       |                      |             |         |            |                       |          |        |                |                       |       |      |  |
| IV/11           | I             | 1 41       | 0 74  | 2 15  | 2 36    | 0 07  | 0 45  | 0 52     | 0 20  | 18 9  | 4 0                  | 22 9        | 30 1    | 5 589      | IV/7                  | 9 9      | 3 3    | III/13<br>IV/8 | -29<br>-29            | IV/6  | 61 4 | Previous basal metabolic r<br>II/20/26, minus 17 per c<br>II/25/26 minus 23 per cen<br>IV/7 low calcium diet |
| IV/15           | II            | 1 18       | 0 53  | 1 71  | 2 97    | 0 13  | 0 32  | 0 45     | 0 19  | 19 6  | 3 9                  | 23 5        | 30 4    | 5 054      | IV/14                 | 10 6     | 3 2    | IV/14          | -36                   | IV/11 | 60 8 | IV/7 low calcium diet  |
| IV/17           | III           | 2 75       | 0 25  | 3 00  | 2 58    | 0 25  | 0 19  | 0 44     | 0 16  | 23 2  | 2 2                  | 25 4        | 25 1    | 5 108      |                       |          |        | IV/15          | -17                   | IV/14 | 61 6 | IV/7 low calcium diet  |
| IV/20           | IV            | 2 22       | 0 43  | 2 65  | 2 13    | 0 27  | 0 29  | 0 56     | 0 20  | 34 9  | 3 5                  | 38 4        | 30 3    | 5 878      | IV/22                 | 10 9     | 3 6    | IV/22          | -5                    | IV/17 | 59 8 | IV/18, thyroxin 5 mgm i m<br>IV/21 thyroxin 5 mgm i m<br>IV/23, thyroid grains iv l d                        |
| IV/23           | V             | 2 59       | 0 35  | 2 94  | 2 28    | 0 41  | 0 26  | 0 67     | 0 23  | 34 8  | 2 7                  | 37 5        | 30 4    | 6 378      | IV/23                 | 10 9     |        |                |                       |       |      |  |
| IV/27           | VI†           | 2 02       | 0 50  | 2 52  | 1 90    | 0 34  | 0 42  | 0 76     | 0 19  | 31 3  | 4 4                  | 35 7        | 25 6    | 5 323      |                       |          |        | IV/27          | -4                    | IV/24 | 58 0 |  |
| IV/29           | VII*          | 0 18       | 2 00  | 2 18  | 0 65    | 0 21  | 0 27  | 0 65     | 0 21  | 16 4  | 1 0                  | 17 4        | 29 6    | 5 425      |                       |          |        | V/12           | +13                   | IV/29 | 58 0 |  |

\* Period VII—Two days and a little over Results multiplied by 3/2 for 3 day period

† Period VI—Lost one small stool and small amount of urine

## Hazel Bancroft W Age 23

|     | VII/23 | VII/26 | VII/29 | VII/23 | 9    | 9    | 4    | 0    | VII/14  | -39 | VII/14  | 52   | 3     |
|-----|--------|--------|--------|--------|------|------|------|------|---------|-----|---------|------|-------|
| I   | 0.57   | 0.40   | 0.97   | 1.18   | 0.16 | 0.31 | 0.47 | 0.28 | 8.2     | 2.2 | 10.4    | 13.8 | 2.960 |
| II  | 0.62   | 0.36   | 0.94   | 1.09   | 0.28 | 0.24 | 0.52 | 0.17 | 11.2    | 2.5 | 14.6    | 13.4 | 1.818 |
| III | 1.64   | 0.46   | 2.10   | 2.90   | 0.49 | 0.34 | 0.83 | 0.22 | 20.1    | 3.2 | 23.3    | 19.4 | 2.609 |
| IV  | 1.78   | 0.37   | 2.30   | 1.21   | 0.57 | 0.39 | 0.96 | 0.23 | 29.6    | 4.8 | 34.4    | 17.1 | 3.236 |
| V   | 1.10   | 0.33   | 1.43   | 1.07   | 0.55 | 0.35 | 0.90 | 0.20 | 21.3    | 3.1 | 24.4    | 14.6 | 2.348 |
|     |        |        |        |        |      |      |      |      | VIII/12 | -9  | VIII/12 | 50   | 7     |

Low calcium diet started on 22nd  
VII/29 started thyroid grains  
vi q daily

## John Francis W Age 63

[illegible]

Elizabeth Mary D Age 52

[illegible]

TABLE 7  
Normal controls

| Dates of period    | Period number | Phosphorus |       |        | Calcium |       |        | Nitrogen |       |          | Total caloric intake | Blood serum    |                |            | Basal metabolic rate |                                   | Treatment and remarks |
|--------------------|---------------|------------|-------|--------|---------|-------|--------|----------|-------|----------|----------------------|----------------|----------------|------------|----------------------|-----------------------------------|-----------------------|
|                    |               | Output     |       | Intake | Output  |       | Intake | Output   |       | Intake   |                      | Date           | Calcium        | Phosphorus | Date                 | Per cent                          |                       |
|                    |               | Urine      | Feces |        | Urine   | Feces |        | Urine    | Feces |          |                      |                |                |            |                      |                                   |                       |
| Clark H Age 25     |               |            |       |        |         |       |        |          |       |          |                      |                |                |            |                      |                                   |                       |
| March 26           |               | gms        | gms   | gms    | gms     | gms   | gms    | gms      | gms   | gms      |                      | mgm per 100 cc | mgm per 100 cc | March 26   | per cent             | March 24, low cal cium supper     |                       |
| March 29           | I             | 3 11       | 1 92  | 5 03   | 3 33    | 0 44  | 0 48   | 0 92     | 0 35  | 54 1     | 45 58 622            | 9 4            | 2 7            | March 30   | -1                   | April 1, thyrold gr xasid         |                       |
| April 1            | II            | 2 45       | 0 92  | 3 37   | 3 33    | 0 47  | 0 41   | 0 88     | 0 35  | 43 1     | 47 1 45 58 622       | 8 1            | 2 8            | April 6    | -5                   | April 7, thyroxin 10 mgm i.v      |                       |
| April 4            | III           | 3 41       | 1 05  | 4 46   | 3 33    | 0 52  | 0 48   | 1 00     | 0 35  | 47 6 5 9 | 53 5 45 58 622       | 10 2           | 3 5            | April 10   | -8                   | April 9, thyrold stopped          |                       |
| April 7            | IV            | 3 74       | 0 85  | 4 59   | 2 76    | 0 53  | 0 36   | 0 89     | 0 37  | 52 0 4 2 | 56 2 45 58 778       |                |                | April 16   | +16                  |                                   |                       |
| April 10           | V             | 3 98       | 0 92  | 4 90   | 2 82    | 0 75  | 0 57   | 1 32     | 0 28  | 56 1 5 3 | 61 4 36 16 939       |                |                |            |                      |                                   |                       |
| April 13           | VI            | 3 44       | 0 95  | 4 38   | 3 53    | 0 75  | 0 61   | 1 36     | 0 38  | 56 4 3 5 | 59 9 48 19 420       | 2 7            | 2 6            |            |                      |                                   |                       |
| April 16           | VII           | 6 25       | 1 31  | 7 56   | 3 53    | 0 73  | 0 74   | 1 47     | 0 39  | 56 7 7 2 | 63 9 48 19 420       | 10 6           |                | April 16   | +48                  |                                   |                       |
| Clement L K Age 27 |               |            |       |        |         |       |        |          |       |          |                      |                |                |            |                      |                                   |                       |
| March 26           |               |            |       |        |         |       |        |          |       |          |                      |                |                | March 23   | -8                   | March 23, a.m , low calcium diet. |                       |
| March 28           | I             | 2 70       | 0 44  | 3 14   | 3 03    | 0 32  | 0 20   | 0 52     | 0 28  | 39 3 2 0 | 41 3 36 06 273       | 9 2            | 2 2            | March 24   | -14                  |                                   |                       |
|                    |               |            |       |        |         |       |        |          |       |          |                      | 8 0            | 2 7            | March 26   | -15                  |                                   |                       |
|                    |               |            |       |        |         |       |        |          |       |          |                      |                |                | March 31   | -12                  | March 31, thyrold gr viid         |                       |

|          |     |      |      |      |      |      |      |      |      |      |     |      |      |       |          |     |          |          |                             |
|----------|-----|------|------|------|------|------|------|------|------|------|-----|------|------|-------|----------|-----|----------|----------|-----------------------------|
| April 1  | II  | 2 39 | 0 92 | 3 31 | 2 74 | 0 38 | 0 43 | 0 81 | 0 28 | 34 8 | 6 9 | 41 7 | 36 0 | 6 273 | April 2  | 9 7 | 3 1      | April 5  | 1                           |
| April 5  | III | 3 05 | 0 54 | 3 59 | 2 74 | 0 41 | 0 25 | 0 67 | 0 28 | 39 1 | 3 9 | 43 0 | 36 0 | 6 273 |          |     |          | --7      | April 5 thyroid gr<br>xvld. |
| April 7  | IV  | 3 24 | 1 01 | 4 25 | 3 12 | 0 37 | 0 28 | 0 65 | 0 30 | 44 3 | 3 7 | 48 0 | 39 6 | 8 820 |          |     | April 10 | +6       | April 9 thyroid<br>stopped  |
| April 10 | V   | 3 20 | 0 69 | 3 59 | 3 19 | 0 59 | 0 36 | 0 93 | 0 31 | 50 8 | 3 2 | 54 0 | 43 6 | 7 519 |          |     |          |          |                             |
| April 13 | VI  | 3 37 | 0 67 | 4 24 | 2 90 | 0 66 | 0 36 | 1 02 | 0 32 | 53 4 | 4 3 | 57 7 | 30 9 | 7 308 | April 13 | 8 3 | 2 3      | April 15 | +2                          |
| April 15 | VII | 2 89 | 0 33 | 3 22 | 2 54 | 0 65 | 0 19 | 0 84 | 0 29 | 49 8 | 2 3 | 52 1 | 40 5 | 6 687 |          |     |          |          |                             |

\*Period IV urine estimated 400 cc. lost on April 1

Urine period stops the morning after carmine has been given--9 a.m.

Feces period stops when carmine stops.

for essentially normal values for calcium and phosphorus are found in the serum

6 X-ray evidence is presented which indicates that marked osteoporosis may develop in the bones from prolonged hyperthyroidism

7 The increased calcium excretion is not dependent upon the elevated metabolism alone, for three out of four cases with high metabolism due to leukemia or fever had normal calcium eliminations

#### PROTOCOLS

Norman G No 273649 Age 29 years, white, male, married Admitted December 17, 1925 Discharged February 28, 1926

*History* One brother had a goiter with hyperthyroidism Patient has been married nine years He has three children One child died at age of 2 months Nine months previous to entry patient noticed nervousness, palpitation, and tremor of extremities Dyspnea and headache were present on exertion There was a gradual progression of symptoms until four months previous to entry when swelling of neck and prominence of eyes was noticed with slightly increased sweating One month previous to entry he had a sense of constriction in his throat and slight constipation He has had nocturia for five or six months

*Physical examination* He was a nervous, restless, sweating, flushed young man with slight prominence of the eyes There was slight lid lag There was a fine tremor of fingers and tongue Tonsils were very large and cryptic Thyroid was symmetrically enlarged, firm and smooth, with definite bruit Heart showed no enlargement There was a systolic murmur over the precordium There was a palpable thrill at the apex Skin was warm and moist

*Laboratory findings* Urine was examined five times—there were a few red blood cells on three occasions and sugar was slightly positive on three occasions, otherwise urine was negative Blood—red count was 4,450,000, white count 7100, hemoglobin 80 per cent Differential count showed polymorphonuclears 45 per cent, small lymphocytes 48 per cent, large lymphocytes 6 per cent, and mast cells 1 per cent Wassermann was negative Non-protein nitrogen was 34 mgm per 100 cc Phthalein excretion was 35 per cent Basal metabolic rate on December 19, 1925 was plus 99 per cent with a weight of 48.3 kgm

*Treatment and progress* He had complete rest in bed and after ten days, Lugol's solution was started On January 8, 1926, he had a sub-total thyroidectomy with uneventful recovery On January 26, 1926, he had an attack of acute tonsillitis with a fever of 104.5 degrees F which lasted ten days On February 3, 1926, he had a tonsillectomy and adenoidectomy performed

*Impression* This was a most severe case of exophthalmic goiter, greatly improved but not cured by operation This condition was complicated by severely infected tonsils

George R A No 275358 Age 24 years, white, male, married, truck driver  
Admitted March 24, 1926 Discharged April 29, 1926

*History* For years he has had frequent frontal headaches For three years he has had shortness of breath and severe palpitation on exertion which has gradually become more intense He also has had an occasional, non radiating, stabbing precordial pain For one year he has noticed an increasing weakness, emotional instability, and a feeling of heat with marked sweating He had a markedly increased appetite but good digestion and regular bowels His neck was growing larger

*Physical examination* He was well developed and nourished Skin was moist There was tremor of the hands There were visible pulsations in his neck Thyroid was enlarged with marked bruit There were no eye signs There was a diastolic murmur heard at the left border of the sternum Blood pressure was 210/75 There was a capillary and Corrigan pulse Heart rate was 130

*Laboratory findings* Urine and blood were normal.

*Treatment and progress* He had complete rest in bed and after two weeks Lugol's solution was started On April 17, 1926, a sub-total thyroidectomy was done He had an uneventful recovery Pathological report showed follicular hyperplasia

*Impression* This was a case of aortic regurgitation with a very mild Graves' disease Operation was advised largely because of the heart lesion and it produced a marked general improvement

Herbert B E No 275575 Age 34 years married, white, male, mail carrier  
Admitted April 5, 1926 Discharged May 23, 1926

*History* He has been married ten years and has three children One child died at 5½ months from lobar pneumonia His wife has liver enlargement and some cardiac decompensation He had a Neisserian infection fifteen years ago For a year he has noticed prominence of his eyes Gradually he noted definite nervousness and irritability, excessive perspiration with a voracious appetite but loss of strength and 22 pounds in weight For two weeks he has noticed a much enlarged thyroid with increased tremor of his hands, palpitation, and dyspnea on exertion He has had three transient attacks of precordial pain in the past month

*Physical examination* He was fairly well developed and nourished and in no distress He was very nervous Skin was moist and warm, with tan pigmentation There was marked bilateral exophthalmos and lid lag Thyroid was symmetrically enlarged There was lumbar lordosis Heart showed a forceful apex impulse but was not enlarged Sounds were rapid and regular Blood pressure was 175/75 There was a fine tremor of the hands

*Laboratory findings* Urine was normal Blood showed a red count of 4,680,000, white count 8,150 and hemoglobin of 75 per cent. Differential count showed polymorphonuclears 61 per cent, small lymphocytes 12 per cent, large lympho-

cytes 25 per cent, and eosinophiles 2 per cent Non-protein nitrogen was 29 mgm per 100 cc Wassermann was strongly positive on two examinations Basal metabolism on April 7, 1926 was plus 48 per cent

*Treatment and progress* He had complete rest in bed and Lugol's solution was started after two weeks On April 29, 1926 a sub-total thyroidectomy was done Pathological report showed follicular hyperplasia Following operation he had typical lobar pneumonia of the left lower lobe with a crisis on the eighth day Basal metabolic rate on May 20, 1926 was minus 18 per cent

*Impression* Exophthalmic goiter, tertiary syphilis

Rose Lee K No 275699 Age 63 years, female, white, married, housewife  
Admitted April 12, 1926 Discharged May 23, 1926

*History* Patient has had fourteen children of whom seven died from obstetrical operations and three died in infancy She has had no miscarriages She has had recurrent tonsillitis She also has had pleuritis and pneumonia She had her menopause at 53 years of age

*Present illness* She was always a hard worker For the past year she had noticed increasing fatigue, nervousness, palpitation, and trembling of hands Swelling in her neck was noticed ten months previous to entry Appetite became poor and she had epigastric distress relieved by food, and difficulty in swallowing For six months she has had shortness of breath and orthopnea She was easily irritated and perspired easily She has lost 57 pounds in weight

*Physical examination* Patient was poorly developed and nourished, apprehensive, and nervous There was slight exophthalmos, blepharitis, and slightly congested pharynx Her thyroid was asymmetrically enlarged, nodular, and very firm There was local glandular enlargement Heart was enlarged to the left Rate was rapid and regular Liver edge was palpable Blood pressure was 180/70

*Laboratory findings* Urine was normal Blood showed red count of 5,200,000, white count 15,000, and hemoglobin 70 per cent Differential count showed polymorphonuclears 63 per cent, small lymphocytes 29 per cent, large lymphocytes 7 per cent, and mast cells 1 per cent Non-protein nitrogen was 26 mgm per 100 cc Wassermann was negative Basal metabolic rate on April 15, 1926 was plus 87 per cent

*Treatment and progress* She had complete rest in bed and after eleven days Lugol's solution was started On May 6, 1926, a sub-total thyroidectomy was performed Pathological report showed follicular hyperplasia Basal metabolic rate on discharge was plus 27 per cent

*Impression* Exophthalmic goiter

Christine B No 274688 Age 22 years, female, white, married, hospital ward maid  
Admitted February 15, 1926 Discharged April 3, 1926

*History* She was separated from her husband She had had no pregnancies She was strong and well up to present illness She noticed a goiter one year

previous to entry and had been easily upset emotionally with crying and nervous spells. For one month she had noticed palpitation, slight prominence of her eyes, increased sweating, and slight difficulty in swallowing.

*Physical examination* She was a well developed and nourished, very short young woman who felt hot. There was definite exophthalmos. There was a fine tremor of hands and tongue. Thyroid was symmetrically enlarged, firm, with bruit. Heart was rapid and regular with no enlargement.

*Laboratory findings* Urine was negative. Blood showed red count 5,656,000, white count 8,000, and hemoglobin 80 per cent. Smear showed polymorphonuclears 78 per cent, small lymphocytes 20 per cent, and large lymphocytes 2 per cent. Non protein nitrogen was 32 mgm per 100 cc. Wassermann was negative. Basal metabolic rate on February 17, 1926 was plus 76 per cent, pulse 116, and weight 43.8 kgm.

*Treatment and progress* Patient had complete rest in bed and Lugol's solution was started at the end of two weeks. On March 12, 1926, a subtotal thyroidectomy was done. Her basal metabolic rate at time of discharge was minus 7 per cent. Patient was apparently cured by the operation.

*Impression* Severe exophthalmic goiter.

Elizabeth B. No 274040. Age 27, female, white, single, stenographer. Admitted January 9, 1926. Discharged March 3, 1926.

*History* Rheumatic fever at eight years for six months. She had two attacks of tonsillitis. Catamenia was somewhat irregular. For ten years she had been nervous, marked by very easy excitability, crying and insomnia. She had grown worse in the last three months and had lost 15 pounds in spite of an increased appetite. She had a feeling of warmth, fatigue, and palpitation. For ten days she had noticed swelling in her neck. She felt quite tired.

*Physical examination* Patient was nervous and twitchy. She was well developed but slightly thin with moist skin. Throat and tonsils were slightly red. There were no definite eye signs. Thyroid showed moderate, smooth, diffuse enlargement greater on the right with a marked bruit. Heart was enlarged. A diastolic murmur was heard by one examiner only. Blood pressure was 144/80.

*Laboratory findings* Urine showed a few white cells, otherwise negative on five examinations. Blood red count 4,300,000, white count 8,000; hemoglobin 70 per cent. Differential count showed polymorphonuclears 50 per cent, small lymphocytes 40 per cent and large lymphocytes 10 per cent. Wassermann was negative. Non protein nitrogen was 29 mgm per 100 cc. Basal metabolism on January 11, 1926, was plus 79 per cent.

*Treatment and progress* Patient had complete rest in bed and Lugol's solution was started at the end of eleven days. During her stay in the hospital she had several attacks of severe pain in her joints accompanied by slight fever and a rise in her white blood count. Joints were all slightly swollen, warm, tender, but not red. She was relieved by salicylates. February 13, 1926, a subtotal thyroidectomy was done. Pathological report showed follicular hyperplasia.

*Treatment and progress* She was greatly improved by the operation and left the hospital apparently well Basal metabolism on February 23, 1926 was plus 7 per cent

*Impression* Moderately severe exophthalmic goiter—complicated by a mild attack of rheumatic fever

Sophia J K No 277495 Age 58 years, married, white, female Admitted July 15, 1926 Discharged August 8, 1926

*History* Patient was in good health until three years ago when she first noticed a lump in the right side of her neck which had gradually increased in size She then became nervous and "fidgety" This increased so that two years ago her hands began to tremble, enough to bother her in writing She also has noticed some palpitation and shortness of breath on the slightest exertion There has been no increased perspiration For the past three years she has had occasional stabbing pains in the lumbar region During the past seven months she has had a vaginal discharge which is watery, at times bloody, and at other times purulent and bloody, no odor She had measles and whooping cough as a child and rheumatism twenty years ago Thirty years ago she was treated for metritis Eleven years ago she had two uterine tumors removed

*Physical examination* She was a nervous woman with marked coarse tremor of hands but no exophthalmos There was a small round hard mass in the right lobe of the thyroid There was a blowing systolic murmur at apex Heart was normal in size Blood pressure was 150/70 She had a moderate cystocele and a marked bloody discharge from the uterus

*Treatment and progress* Operation was performed with excision of adenoma Dilatation and curettage was done, the curettings showing tuberculosis

*Impression* Toxic adenoma of thyroid, tuberculous endometritis

Margaret D No 269527 Age 48 years, white, married, female Admitted April 8, 1926 Discharged May, 13, 1926

*History* She has been married twice First husband divorced She has one child living and well Menopause occurred at 44 years Patient entered the hospital one year previous to present entry complaining of nervousness of 8 months duration, some dizziness and dyspnea, increased perspiration, dislike of warm places, slight palpitation for one month, with a tight feeling in her throat She had noticed enlargement of the thyroid for some time She refused operation at that time Two treatments by x-ray were without benefit and she feels she has grown worse since her first entrance Dyspnea and weakness are increased She is emotionally unstable and she has noticed an increase in the size of her neck with some difficulty in swallowing

*Physical examination* She was a well developed and fairly well nourished woman with considerable brownish pigmented areas of the skin There was moderate exophthalmos and some tremor of fingers The right lobe of the

thyroid had a solid, nodular enlargement, approximately 5 by 5 cm with a left lobe not appreciably enlarged Heart showed no enlargement There was a systolic murmur at the aortic area Blood pressure was 124/80

*Laboratory findings* Urine was negative Blood was negative Wassermann was negative May 10, 1926, non protein nitrogen was 26 mgm per 100 cc

*Treatment and progress* She had complete rest in bed and Lugol's solution was started after seven days April 28 1926 a lobectomy was performed Pathological report 'Probably comes within limits of normal thyroid except for few miliary adenomata' She made an uneventful recovery Patient was very quiet and calm when discharged Basal metabolic rate on discharge was plus 5 per cent

*Impression* Adenoma of thyroid with mild toxicity

John Francis W No 277489 Age 63 years, widower Admitted July 15, 1926 Discharged August 14, 1926

*History* He dated his present pallor back twenty years He said it came on following a "drinking bout" and had been present ever since He thought his skin was more dry coarse, and yellow than formerly Several years later he noticed his body hair (eyebrows, chest, axillary and pubic) began to fall out For the past three years he had noticed he could not stand cold weather He had had increasing weakness and inability to endure exercise He was drowsy most of the time and said his memory was very poor for recent events He had noticed increased constipation for the past few years Appetite was fair He had had puffy eyes of late He could not obtain work as a collector because of his appearance There had been no increase in weight

*Physical examination* He was a very obese man of 63 with a marked pallor of his mucous membranes Skin had a yellow tint, was coarse, dry and appeared edematous—did not pit on pressure His eyebrows were almost absent, as was the hair of his axillae chest and pubic region His tongue was thick Speech was slow and sluggish There was a perforation of the nasal septum Lungs showed chronic bronchitis Heart was negative Blood pressure was 160/74 Vessels showed moderate sclerosis

*Laboratory findings* Urine blood and Wassermann were all negative Non protein nitrogen on July 15, 1926 was 30 mgm per 100 cc

*Progress and treatment* Patient was studied for 14 days before therapy was started On July 31, 1926 he was started on thyroid extract grains 6 per day On thyroid therapy he was greatly improved Basal metabolic rate on August 8, 1926 was minus 14 per cent

*Impression* Myxedema, arteriosclerosis, emphysema and chronic bronchitis

Hazel B W No 277422 Age 23 years, single, white, female Admitted July 12 1926 Discharged August 12, 1926

*History* For years patient had suffered from headache which was relieved by rest For the past eight months she had had increasing general weakness often

accompanied by shortness of breath requiring rest after she had walked a few hundred yards. For the past six months she had been sensitive to cold, in fact she felt cold most of the time. She also had noticed she perspired very little and that her skin was dry and much coarser than previously. During this same period her memory had become poor. At times her tongue had felt swollen and tender. With the above symptoms she had increased gradually in weight so that at the time of entry she weighed 130 as compared with her best previous weight of 102. Appetite was poor. She had been constipated for years. Catamenia had been absent for the past 2½ years.

*Physical examination* She was a young woman of 23, well developed and nourished. She appeared drowsy and apathetic. Face had a puffy appearance especially about the eyes. Skin was coarse and dry. Hair was coarse. She had a definite pallor. Blood pressure was 80/60.

*Laboratory findings* X-rays showed an enlarged sella turcica and sacroiliac arthritis. Electrocardiogram showed the T<sub>2</sub> to be 1 mm with small complexes and a rate of 85. Wassermann was negative. Blood—red count was 3,720,000, white count 7,500, hemoglobin 80 per cent. Non-protein nitrogen was 26 mgm per 100 cc. Phthalein excretion was 5 per cent.

*Follow-up notes* September 11, 1926. She did not mind the cold. Her strength seemed to have returned. Appetite was better. She talked much faster. Her skin was softer. She weighed 108 pounds. She was taking thyroid grains 6 daily. Basal metabolic rate was plus 35 per cent. Thyroid was cut down to grains 2 per day because of the high basal metabolic rate.

November 16, 1926. Menses returned last month, lasted seven days, required three to four napkins per day. It was non-painful. Her basal metabolic rate was plus 1 per cent but she had some symptoms of myxedema returning. Thyroid was increased to grains 4½ per day.

*Impression* Myxedema

Elizabeth M. D. No 276291. Age 52 years, white, married, female. First admission May 13, 1926. Discharged May 20, 1926. Second admission June 2, 1926. Discharged July 2, 1926.

*History* She had her menopause seven years ago. She felt well until one year ago. At that time numbness and a cold feeling in her hands began. She gained 28 pounds in one year. She had minded the cold especially during the past winter. Her skin always had been dry and she had not perspired much. Her hands had become broad and puffy in the past two years. She was not excitable and her memory had become poor. There had been very little loss of hair. Her appetite was good. She had no dyspnea and no palpitation. She had recently stumbled over curbstones and for six months she had had difficulty in climbing stairs because of stiffness of the legs.

*Physical examination* She was a well developed and nourished, obese woman—dull and apathetic. Skin was coarse and dry. Hair was rather coarse. Heart

was not enlarged. The sounds were distant. There was a soft systolic murmur at the base. Abdomen was protuberant with diastasis recti. Extremities showed varicose veins and slight edema of both ankles.

*Laboratory findings.* Urine was negative except for occasional white blood cells and epithelial cells on four examinations. Blood—red count was 4,300,000. White count was 6,400. Hemoglobin was 60 per cent. Differential count showed polymorphonuclears 64 per cent, small lymphocytes 30 per cent and large lymphocytes 6 per cent. Non-protein nitrogen was 38 mgm. per 100 cc. Wassermann was negative. Basal metabolic rate was minus 34 per cent. Weight was 73.5 kgm.

*Treatment and progress.* She had complete rest in bed and during this time metabolic studies were made. She was discharged on May 20th because of death in her family. No treatment was started.

She returned, June 2, 1926. She was put on a low calcium diet and calcium metabolism studies were made before and during thyroid therapy.

*Impression.* Myxedema.

Mable H. M. No. 274766. Age 34 years, white, married, female. First admission February 19, 1926. Discharged February 25, 1926. Second admission April 6, 1926. Discharged April 28, 1926.

*History.* She had been married eight years. She had one child. She had had no miscarriages. For ten years she had been troubled with a dull ache in her lower back on bending forward and had felt tired. For five years this weakness and fatigue had become increasingly more severe. She noticed slowing of her mental reaction and a feeling of coldness. Catamenia was always irregular and painful. She was nervous, irritable, and slept poorly. Three and a half years ago her basal metabolic rate at the Homeopathic Hospital was minus 15 per cent and minus 18 per cent. For two years she had noticed thickness of her tongue and lips with difficulty in her speech. "Thyroid extract" at first resulted in headaches and palpitation but gave her relief.

*Physical examination.* She was well developed and nourished, with thick lips and tongue. The skin of her face was slightly yellowish. Expression was slightly apathetic. She had external hemorrhoids.

*Laboratory findings.* Urine was negative. Red blood count was 4,384,000, white count 8,600, hemoglobin 80 per cent. Differential count was normal. Non-protein nitrogen was 35 mgm. per 100 cc. Wassermann was negative. Basal metabolic rate on February 20, 1926, was minus 17 per cent. Basal metabolic rate on April 8, 1926, was minus 29 per cent. Fundi were negative. X-ray showed a large deep sella turcica.

*Progress.* This patient was very much improved by thyroid therapy but was still nervous and easily upset emotionally. She tired very easily and had frequent frontal headaches.

*Impression.* Mild myxedema. Question of pituitary tumor; sacro iliac arthritis.

Jennie W No 270359 Age 46 years, white, single, female History of previous entry—June, 1925

*History* Two years ago she came to the Out Patient Department complaining of weakness, lack of ambition, loss of appetite and coldness of extremities These symptoms had come on gradually over two years Her speech was dull and listless and she looked pale with pouches under her eyes Menopause occurred six years ago Basal metabolic rate on July 3, 1924, was minus 26 per cent Basal metabolic rate on July 22, 1924, was minus 32 per cent She was given thyroid extract—grains  $1\frac{1}{2}$  three times a day Basal metabolic rate on August 11, 1924 was minus 13 per cent Thyroid was increased to  $7\frac{1}{2}$  grains a day Basal metabolic rate on September 5, 1924, was minus 1 per cent and patient felt better Basal metabolic rate on January 26, 1925, was minus 24 per cent Thyroid was reduced Basal metabolic rate on April 10, 1925, was minus 13 per cent She then entered the Hospital (June, 1925) and her condition was studied Discharged July 4, 1925

*Second admission* was on April 30, 1926 She returned because of recurrence of symptoms After discharge she was taking  $7\frac{1}{2}$  grains of thyroid daily but her supply gave out about one month ago and symptoms slowly recurred Basal metabolic rate on April 27, 1926, was minus 33 per cent

*Physical examination* She was well developed and nourished with slight puffiness under her eyes There was moderate pallor of her skin with very slight dryness

*Laboratory findings* Urine was negative Blood showed a red count of 4,216,000, white count 5,700, and a hemoglobin of 65 per cent Differential count showed polymorphonuclears 38 per cent, small lymphocytes 47 per cent, large lymphocytes 4 per cent, mast cells 9 per cent and eosinophiles 2 per cent Non-protein nitrogen was 23 mgm per 100 cc Basal metabolic rate was minus 34 per cent Wassermann was negative

*Treatment and progress* She had complete rest in bed On May 14, 1926, she developed a definite psychosis with delusions of persecution She wanted to leave the hospital She was given thyroxin, 10 mgm intramuscularly On May 16, 1926, her psychosis was much improved, 48 hours after 10 mgm of thyroxin intramuscularly, yet she retained certain ideas of persecution The basal metabolic rate rose to plus 13 per cent on thyroid extract

*Impression* Myxedema complicated by mild psychosis

Susan B F No 265471 Age 48 years, white, married, female Admitted June 1, 1926 Discharged July 15, 1926

In 1924 she was in the Massachusetts General Hospital with the diagnosis of (1) strangulated internal hernia, (2) myxedema, (3) chronic cystitis A laparotomy for intestinal obstruction was done

*History* She was born and had lived in Halifax, N S, until 7 years ago She had nine children and no miscarriages Her periods stopped when her last child

was born 17 years ago. She had not felt well since then. At that time she suffered from weakness and shortness of breath and often had to rest. She noticed some paleness and bloating of her skin with considerable drowsiness. After two years of these symptoms treatment with "pills and medicine" relieved her and she remained fairly well until 1918 when she had influenza, after which she became weaker and more short of breath on exertion. No other symptoms occurred until two or three years ago when a new group appeared. Her face became more bloated, lid fissures narrowed, at times her eyes almost closed and she had swelling of her hands and ankles. Her skin was rough, pale, dry and flaked readily and increased thickness was especially noticeable over the extremities. Her hair fell out, was coarser and more brittle. Her feet have been cold during the past three or four years. At times she had tingling and numbness of her fingers and toes. Her speech was more indistinct, slower and her tongue felt thick. Her memory was bad, she remembered past events better than present. She had frequent dizzy spells. Her appetite was poor. She had been constipated for years. Occasionally she had short transient pains over precordium accompanied by smothering and choking spells. All symptoms had grown progressively worse so that she needed a three to four day rest in bed every week. Apparently she had not taken the thyroid extract which had been prescribed for her.

*Physical examination* She was a pale, sluggish woman with well marked edema about the eyes. Her skin was dry. Her hair was coarse and brittle. Tongue was large. Mucosae were pale. Heart was slightly enlarged to the left. Sounds were feeble and distant. There was a moderate amount of pitting edema over the vertebral column from first lumbar vertebra down. Knee jerks were extremely sluggish. There was a reddish brown scar of an old varicose ulcer, over the middle anterior surface of the left tibia.

*Laboratory findings* Urine was negative except for occasional white blood cells and hyaline casts on three examinations. Blood showed a red count of 3,088,000, white count 3,160, hemoglobin 45 per cent. Differential count showed polymorphonuclears 44 per cent, small lymphocytes 46 per cent, large lymphocytes 1 per cent, mast cells 4 per cent, basophiles 2 per cent, and eosinophiles 3 per cent. Platelets were increased. Non protein nitrogen was 38 mgm per 100 cc. Basal metabolic rate was minus 35 per cent, weight 60.4 kgm.

*Treatment and progress* She had complete rest in bed.

June 7, 1926 Patient was very weak. She complained of weakness, pains in elbows and knees. She seemed semi stuporous at times. She had nausea and vomiting. Patient was given 10 mgm thyroxin at 10 15 p m.

June 10 1926 Patient had sore throat with fever. Thyroxin 10 mgm given.

June 13, 1926 Patient had very low caloric intake and continued fever. She complained of pain in the muscles of arms and legs. Thyroxin 10 mgm again given.

June 22 1926 She was weaker and very nauseated. She ate nothing and refused to cooperate.

June 26, 1926 She seemed disoriented and irrational at times Non-protein nitrogen 80 mgm per 100 cc Serum phosphorus 8.8 mgm per 100 cc White blood count was 4100 She was given 20 cc of  $\text{CaCl}_2$  intravenously

June 28, 1926 She was given 20 cc  $\text{CaCl}_2$  again and 500 cc 5 per cent dextrose intravenously Non-protein nitrogen today was 110 mgm per 100 cc At times she was irrational, at other times semi-comatose

July 14, 1926 She was much improved, was eating again, and did not vomit her food She looked thin The signs of myxedema had disappeared

*Impression* The most marked type of myxedema

### BIBLIOGRAPHY

- 1 Koeppen, H, Neurologisches Centralblatt, 1892, xi, 219 Ueber Knochenerkrankungen bei Morbus Basedowni
- 2 Pierallini, G, Lo Sperimentale (Archivio di Biologia normale e patologica) 1906, lx, 59 Sull eliminazione della calce e della magnesia in rapporto ad alcune forme cliniche
- 3 Scholz W, Ztschr f exper Path u Therapie, 1905, ii, 271 Ueber den Stoffwechsel der cretinen
- 4 Silvestri, T and Tossati, C, Gaz degli Ospedali, 1907, xxviii, 1067 Di una funzione della glandola tiroide non ancora ben studiata
- 5 Falta, W, Wien klin Wochnschr, 1909, xxi, 1059 Weitere Mitteilungen uber die Wechselwirkung der Drusen mit innerer Sekretion
- 6 Parhon, M, Memoires de la Societe de Biologie 1912, lxxi, 620 L'influence de la thyroide sur le metabolisme du calcium
- 7 Kojima, M, Quart J Exper Physiol, 1917, xi, 351 Studies on the Endocrine Glands, V Effects on Metabolism of Castration, of Thyroidectomy, of Parathyroidectomy and of Thyroid and Parathyroid Feeding
- 8 Kummer, R H, Rev med de la Suisse Romande, 1917, xxxvii, 439 Recherches sur le metabolisme mineral dans la maladie de Basedow
- 9 Vines, H W C, The Parathyroid Glands in Relation to Disease London, 1924, E Arnold and Co
- 10 Bauer, W and Aub, J C, J Am Diet Assoc, 1927, III, 106 Studies of Inorganic Salt Metabolism I The Ward Routine and Methods
- 11 Deuel, H J, Jr, Sandiford, I, Sandiford, K, and Boothby, W M, J Biol Chem, 1928, lxxvi, 407 The Effect of Thyroxin on the Respiratory and Nitrogen Metabolism on a Normal Subject Following Prolonged Nitrogen-free Diet
- 12 Bauer, W, Albright, F, Rossmeisl, E, and Aub, J C, Studies of Calcium and Phosphorous Metabolism VI In Parathyroid Tetany To be published
- 13 Plummer, W A, and Dunlap, H F, Proc Staff Meetings of Mayo Clinic, 1928, iii, 119 Cases Showing Osteoporosis due to Decalcification in Exophthalmic Goiter

- 14 Goto, K., J Biol. Chem., 1918, xxxvi, 355 Mineral Metabolism in Experimental Acidosis.
- 15 Hunter, D , and Aub, J C., Quart. J Med., 1927, xx, 123 Lead Studies XV The Effect of the Parathyroid Hormone on the Excretion of Lead and of Calcium in Patients Suffering from Lead Poisoning
- 16 Greenwald, I , J Biol Chem , 1926, lxxvii, 1 The Effect of the Administration of Calcium Salts and of Sodium Phosphate upon the Calcium and Phosphorus Metabolism of Thyro-Parathyroidectomized Dogs, with a Consideration of the Nature of Calcium Compound of the Blood and their Relation to the Pathogenesis of Tetany
- 17 Farquharson, R , Salter, W , and Aub, J C. To be published



# STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

## IV THE EFFECT OF THE PARATHYROID HORMONE<sup>1</sup>

BY FULLER ALBRIGHT,<sup>2</sup> WALTER BAUER,<sup>2</sup> MARION ROPES AND  
JOSEPH C AUB

*(From the Medical Clinic of the Massachusetts General Hospital, Boston)*

(Received for publication September 14 1928)

In our studies of calcium and phosphorus metabolism considerable data have accumulated on the physiological effect of parathormone (Collip's preparation) on normal and pathological subjects We are presenting in this paper such of these data as seem to us of value in explaining its action

As explained in a previous paper (1) of this series, we employ a diet inadequate in calcium when studying the effect of various factors on the endogenous calcium metabolism In a control series of thirteen "normal" male individuals with an average age of 41 years, an average weight of 62 kgm, and an average calcium intake of 0.33 gram per three-day period, there was an average output in the urine of 0.19 gram, in the feces of 0.60 gram, making a total average output of 0.79 gram, and an average negative calcium balance per three day period of 0.46 gram (1) In the observations about to be described we have studied the quantitative effect of parathormone injection on this negative calcium balance and have as controls both the series summarized above and periods without medication on each individual subject The methods employed in the preparation of the diet, the collection of the excreta, and the chemical analysis of the material have been described in a previous paper (2)

*Case I* This case is presented first because it is in every way characteristic and because it presents the minimum number of

<sup>1</sup> This investigation was aided in part by the Lead Fund of the Harvard Medical School and by the Fund for the Study of Otosclerosis

<sup>2</sup> Research Fellow, Massachusetts General Hospital and Harvard Medical School

<sup>3</sup> Medical Resident, Massachusetts General Hospital

complicating factors The subject was a girl of 16 years whose sole abnormality was otosclerosis of several years duration We have no reason to suppose that otosclerosis affects the action of parathormone, although it may have some quantitative effect on the negative calcium balance while the subject is on a low calcium diet (3) She was studied for twelve three-day periods on a low calcium diet There was a three-day period on low calcium diet before the investigation was started, and this practice was adhered to in the other investigations reported in this paper She received parathormone injections from the third to the eighth periods inclusive In chart Ia and Ib are given the data of the calcium, phosphorus, and nitrogen balances, and of the blood serum calcium and phosphorus

From the data the following observations of the effect of parathormone upon a subject receiving a low calcium diet are noted

(i) During parathormone administration there is an increase in the negative calcium balance (in this case 165 per cent)

(ii) Following cessation of parathormone administration, the negative calcium balance is less than in the control periods, suggesting a compensatory mechanism

(iii) The calcium excretion in the feces is unaffected by parathormone administration

(iv) During parathormone administration the serum calcium rises The increased urinary calcium excretion is roughly parallel to this rise

(v) Both the rise in the urinary calcium on administration of parathormone and the fall in the urinary calcium on cessation of parathormone tend to be gradual (The rise is unusually abrupt in this case)

#### THE PHOSPHORUS METABOLISM

The calcium losses from the body are at the expense of the calcium deposited in the bones This calcium is largely deposited as tertiary calcium phosphate ( $\text{Ca P } 1.93:1$ ), but also partly as calcium carbonate, so that the ratio of calcium to phosphorus in bone is approximately  $2.23:1$  Therefore, it is of interest to determine whether phosphorus excretion during parathormone injection corresponds to the increased calcium excretion If such were the case and all other fac-

tors were constant, the ratio of the negative calcium balance to the negative phosphorus balance should be 1.931 or 2.231, depending on whether parathormone affects only the calcium in calcium phosphate or whether it affects both the calcium in calcium phosphate and the calcium in calcium carbonate. As the difference between these two factors is less than the limit of error of our data, we have arbitrarily chosen the Ca/P factor as 2.231. Before a theoretical phosphorus balance thus obtained can be compared with the actual phosphorus balance, a correction has to be made for the phosphorus involved in the deposition or liberation of protein ( $N/P = 17.4$ ). Therefore, the phosphorus equivalent of the nitrogen balance and the phosphorus equivalent of the calcium balance have been calculated. The sum of these equals the "theoretical phosphorus balance," it being assumed that the phosphorus in muscle, body fluids, etc., would tend to remain in equilibrium. When we later have occasion to use the expression "theoretical phosphorus excretion" we mean the phosphorus intake plus the "theoretical phosphorus balance." It should be noted that since the phosphorus intake is relatively high and since there must be considerable error in its estimation, the determined phosphorus balance is apt to vary from the theoretical by a certain fixed amount. Since the diet is constant throughout the experiment, this error will be constant. In comparing the "theoretical phosphorus balance" with the actual phosphorus balance, the relative changes from period to period are therefore more significant than the actual difference in the two balances in any one period. In order to study these balances, we have constructed chart Ic. Here the actual and theoretical phosphorus balances have been recorded.

From an inspection of the phosphorus data (chart Ib), we have the additional observations

(vi) The urinary phosphorus is increased by parathormone administration without an effect upon the nitrogen balance

(vii) The fecal phosphorus is little if at all affected by parathormone injection

(viii) The serum phosphorus is decreased during parathormone administration

(ix) As the serum phosphorus falls during parathormone administration, the serum calcium rises

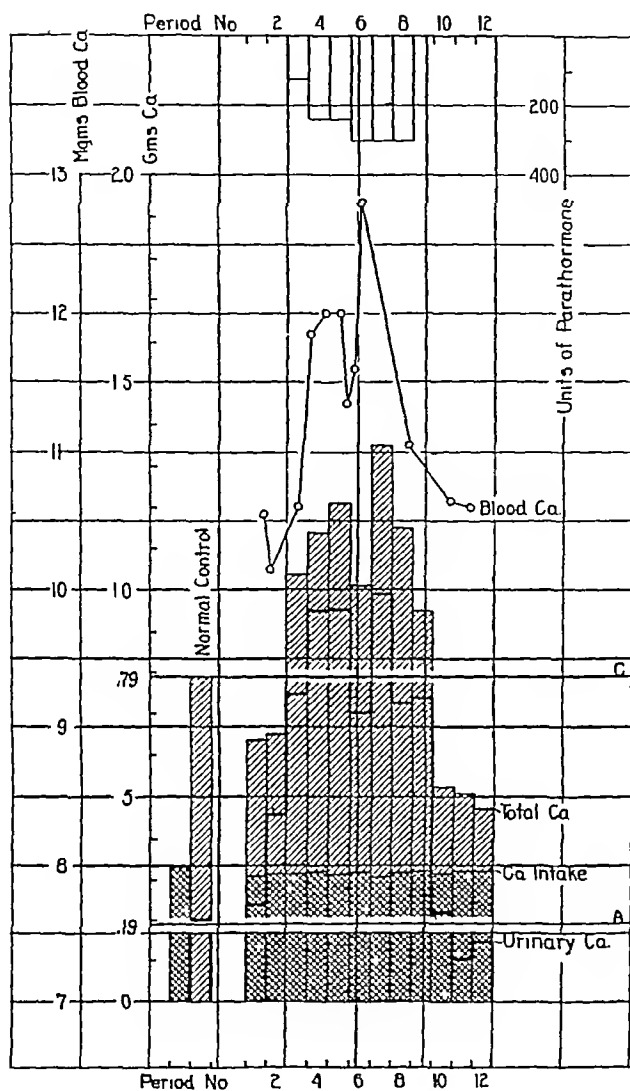


CHART Ia CALCIUM METABOLISM IN CASE I

Line C represents average total calcium excretion of control series on a low calcium diet. Line A represents average urinary calcium excretion of control series on a low calcium diet (1). These lines have the same significance in later charts.

(x) The urinary phosphorus excretion tends to rise abruptly, often to its highest point during the first period of parathormone administration, and to fall abruptly to the pre parathormone level in

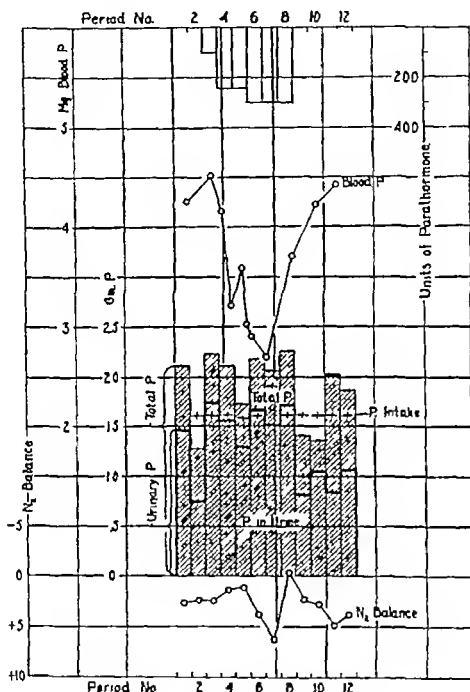


CHART 1b PHOSPHORUS METABOLISM IN CASE I

the first period following cessation of parathormone. This is in contrast with the urinary calcium excretion which shows a latent period.

A careful examination of chart 1c shows a relationship between the theoretical and calculated phosphorus balances. This seems hit or miss on first inspection but will be found constant in all later experi-

ments, namely, that there is a *phosphorus excretion in excess of the theoretical amount during the first period or periods of parathormone administration*, and a *phosphorus excretion less than the theoretical amount during the first period or periods following cessation of parathormone*

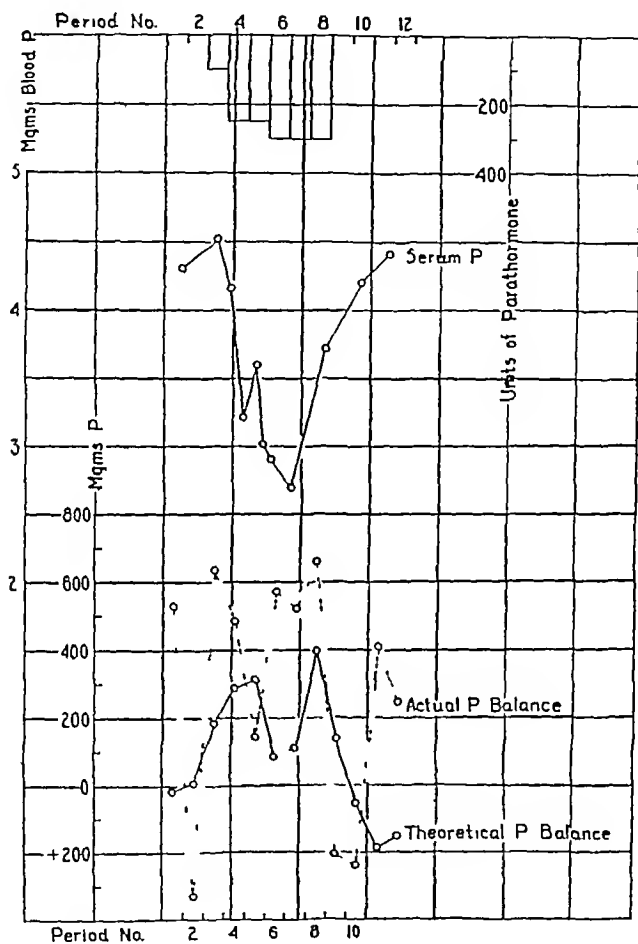


CHART Ic "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE I

One can predict variations in the urinary calcium excretion for any given period from chart Ic by the relationship of the theoretical phosphorus balance to the calculated phosphorus balance of the preceding period. Thus periods 3 and 6, where the actual phosphorus excretion

tions rise much more than the theoretical phosphorus excretions, are followed by a rise in the urinary calcium in periods 4 and 7, periods 5 and 9, where the actual phosphorus excretions fall much more than the theoretical phosphorus excretions, are followed by a fall in the urinary calcium in periods 6 and 10

It will now be necessary to consider the significance of an actual phosphorus excretion in excess of a theoretical phosphorus excretion. One of two explanations seems likely. Possibly when calcium phosphate is mobilized from the bones the phosphorus is excreted more rapidly than the calcium. Evidence against this explanation is found in case VI of this paper. Or possibly it may be due to an excretion of the phosphorus in solution in body fluids, blood, etc. If such is the case one would expect the serum phosphorus to vary with the discrepancy between the actual and theoretical phosphorus excretions. In chart Ic, it will be noted that this appears true. We have then these further observations:

(xi) During the first period of parathormone administration more phosphorus is excreted than can be explained by the calcium and nitrogen balances (theoretical phosphorus excretion)

(xii) During the first period following cessation of parathormone administration, less phosphorus is excreted than can be explained by the calcium and nitrogen balances

(xiii) During parathormone administration any tendency for the actual phosphorus excretion to rise above the theoretical phosphorus excretion is followed by a tendency for the urinary calcium excretion to rise after a latent period of about 3 days (1 period). The converse is likewise true

(xiv) A rise in the actual phosphorus excretion above the theoretical phosphorus excretion is apparently partly at the expense of the phosphorus in the body fluids and results in a fall of the serum phosphorus

From case I we have made certain observations. These are confirmed by the other cases and new observations are added

*Case II* This case is very similar to case I. The subject was a boy of 16 who had symptoms of otosclerosis for about four years. He was studied for thirteen three-day periods on a low calcium diet, then sent home for three months on a normal diet, and finally further studied on a low calcium diet for fifteen more three-day periods. The

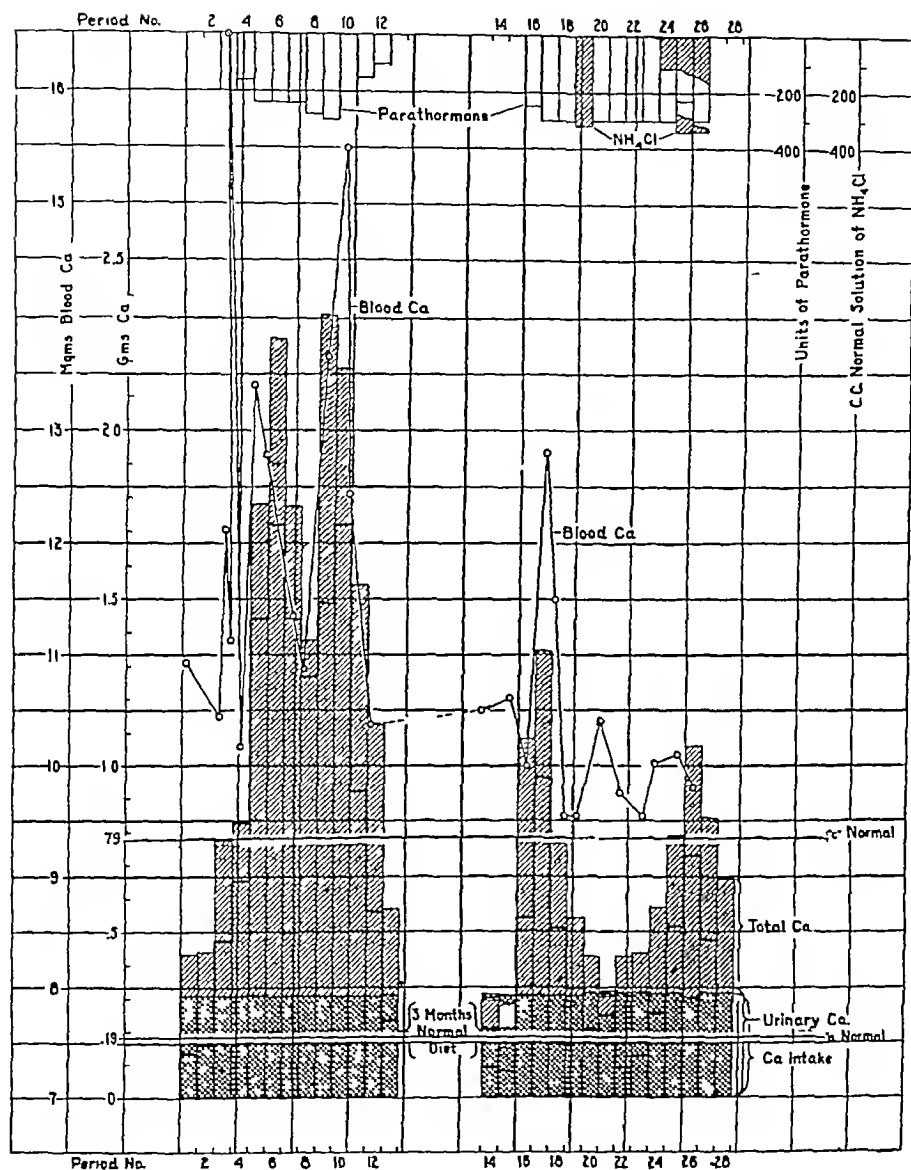


CHART IIa CALCIUM METABOLISM IN CASE II

medication and the calcium, phosphorus and nitrogen balances are recorded in charts IIa, IIb and IIc

The calcium data confirm the observations made on case I. The calcium rise (periods 3, 4, and 5) is more step like than in case I. The negative calcium balance during the control periods (1, 2, 14,

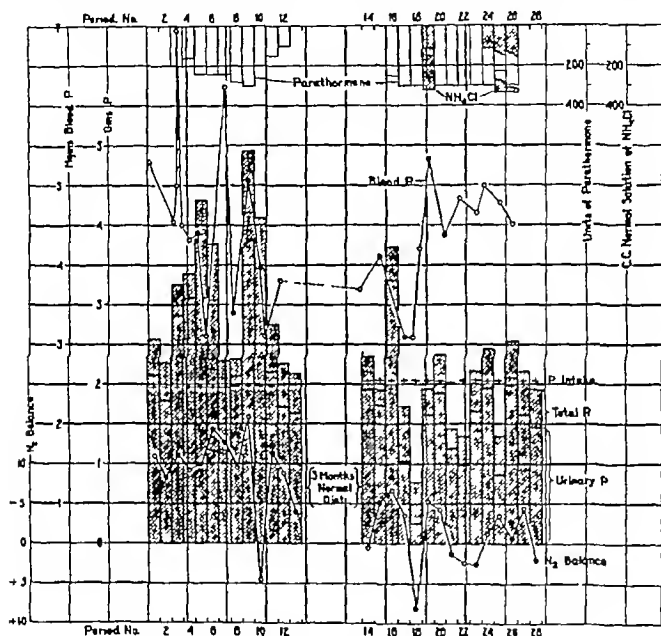


CHART IIb PHOSPHORUS METABOLISM IN CASE II

and 15) is very low, and actually becomes positive in periods 14 and 15. Whether this is due to the fact that the patient was growing or to the fact that he had otosclerosis has not been determined and does not affect the present discussion. The very high serum calcium in period 3 represents a determination taken in the afternoon and so is not com-

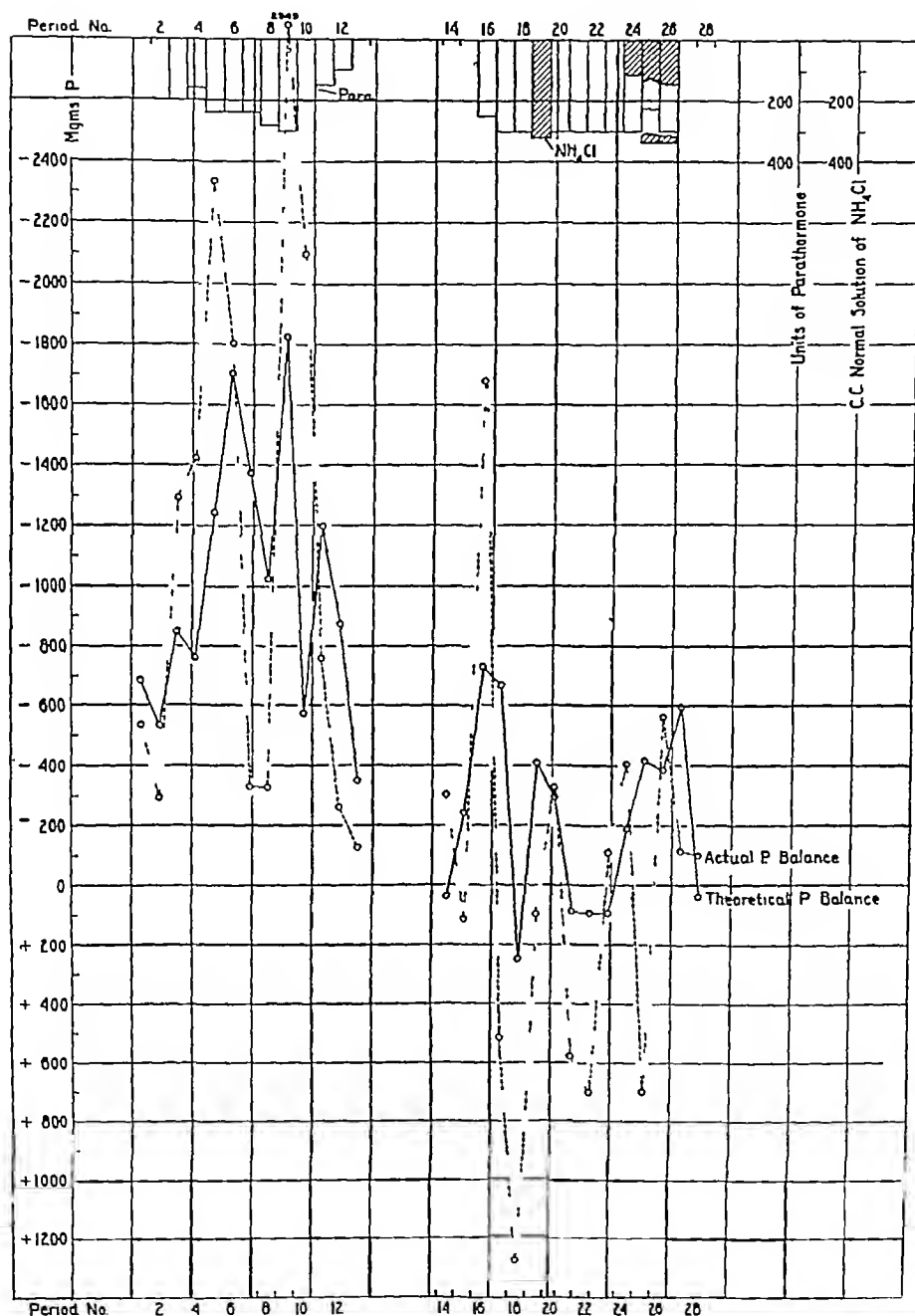


CHART IIc "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE II

parable with the other calcium determinations which represent morning values. This is important because the serum calcium level after a dose of parathormone varies over a period of hours, so that in a study like this the injections and the blood examinations should be made at a fixed time each day. It should be noted that the calcium response to parathormone was much greater in this than in the previous case. In the second half of the experiment, the response to parathormone, after a satisfactory start in periods 16 and 17, completely wears off in periods 18 to 23. This is apparently not due to a lack of available calcium as there is a good response to ammonium chloride in periods 24 to 26. The calcium response to ammonium chloride, like the calcium response to parathormone, is almost entirely urinary.

The phosphorus data (charts IIb and IIc) also corroborate the observations on case I. Parathormone affected the phosphorus excretion more abruptly than it did the calcium. The initial rise in the first periods of medication and the rapid return to normal on cessation of the drug is more precipitous in the phosphorus figures. This is brought out more clearly when comparing the actual phosphorus output with the theoretical figure. In the first response to parathormone the actual phosphorus excretions exceed the theoretical expectations, and are less than the theoretical expectations when the parathormone response disappears. Phosphorus excretions above the theoretical appear to be associated with a falling blood phosphorus and to be accompanied in the following period by a rising calcium excretion. During the second admission the same principles prevail, namely, the actual phosphorus excretion exceeds the theoretical phosphorus excretion during the first period of parathormone administration and is accompanied by a falling serum phosphorus, then as the parathormone becomes ineffective the actual phosphorus excretion falls below the theoretical phosphorus excretion and the serum phosphorus rises to an unusually high level. The effect of the ammonium chloride on the phosphorus excretion is rather inconclusive. The tendency seems to be for the urinary phosphorus to rise, which was to be expected (4) (11) (see also cases III and VI below).

The additional observations to be noted from case II are

(rv) The effect of parathormone injection on the urinary calcium

excretion and the blood serum calcium may wear out completely. This has been corroborated by our studies of tetany (5).

(xvi) This impotency of parathormone is apparently not due to an exhaustion of the available supply of calcium, because the calcium excretion can still be increased in a normal manner by ammonium chloride ingestion.

(xvii) The first evidence of a cessation of the effectiveness of parathormone is a fall in the actual phosphorus excretion below the theoretical phosphorus excretion.

(xviii) With ammonium chloride ingestion there is a rise in urinary calcium and phosphorus excretion.

*Case III* In case III, as in case II, we have further opportunity of studying what happens as parathormone injections lose their physiological effect. The subject was a colored boy of 14 who was suffering from an ossifying hematoma (myositis ossificans) of the right thigh, which had developed as a result of trauma three weeks before admission. In order to see whether parathormone could mobilize calcium from regions other than the bones, we gave the patient a low calcium diet with parathormone injections. No metabolism studies were made until the patient had been on this regime for 16 days, though there was obviously a fair response to parathormone as judged from the fact that the blood serum calcium rose to a maximum of 13 mgm. The patient was then studied for 20 three-day periods while still on a low calcium diet. He was sent home for four months on a normal diet, and finally was studied again for 9 three-day periods on a low calcium diet. The medication and the calcium, phosphorus, and nitrogen data are given in charts IIIa, IIIb, and IIIc. During period 2 the patient had an attack of tonsillitis but his temperature remained elevated only 24 hours. He developed generalized urticaria during period 25, which cleared up promptly when parathormone was discontinued. All of these efforts to eliminate body calcium produced no change in the density of the ossifying hematoma as demonstrated by x-ray.

An inspection of chart IIIa shows that in spite of increasing doses the effect of parathormone on the serum calcium and urinary calcium excretion was gradually lost. It is to be noted especially that the calcium excretion falls not only to what it would be without parathor-

none but even to a much lower level, so that finally a positive balance is obtained in the latter half of the observation

The second part of this observation follows a rest period of four months. During this time he grew very markedly, and this may be

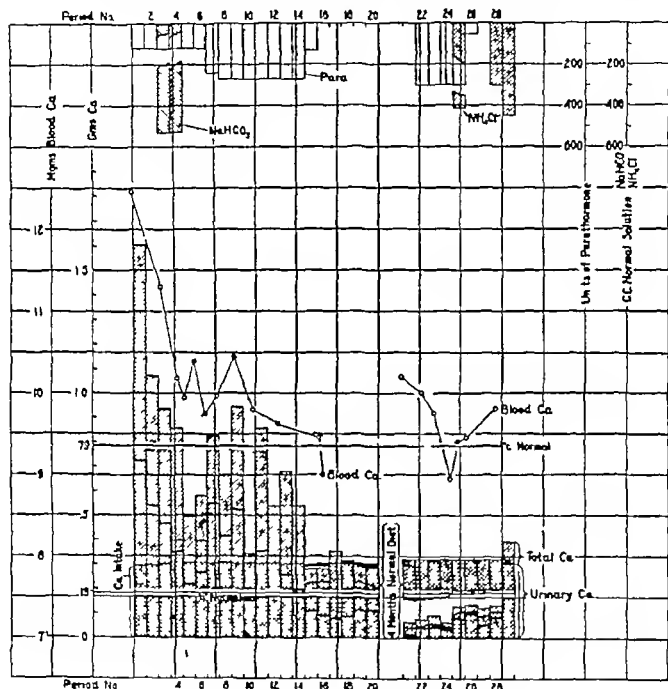


CHART IIIa. CALCIUM METABOLISM IN CASE III

the cause for his having the lowest calcium excretion we have observed in normal individuals on this test diet. In this second observation large doses of parathormone again fail to affect the calcium excretion, and the blood calcium even falls, although ammonium chloride still

proved effective. Thus the observation is corroborated that mobilization of calcium can still be accomplished though parathormone proves ineffective.

The effect of parathormone on the phosphorus metabolism is similar in kind to that seen in cases I and II. On his second admis-

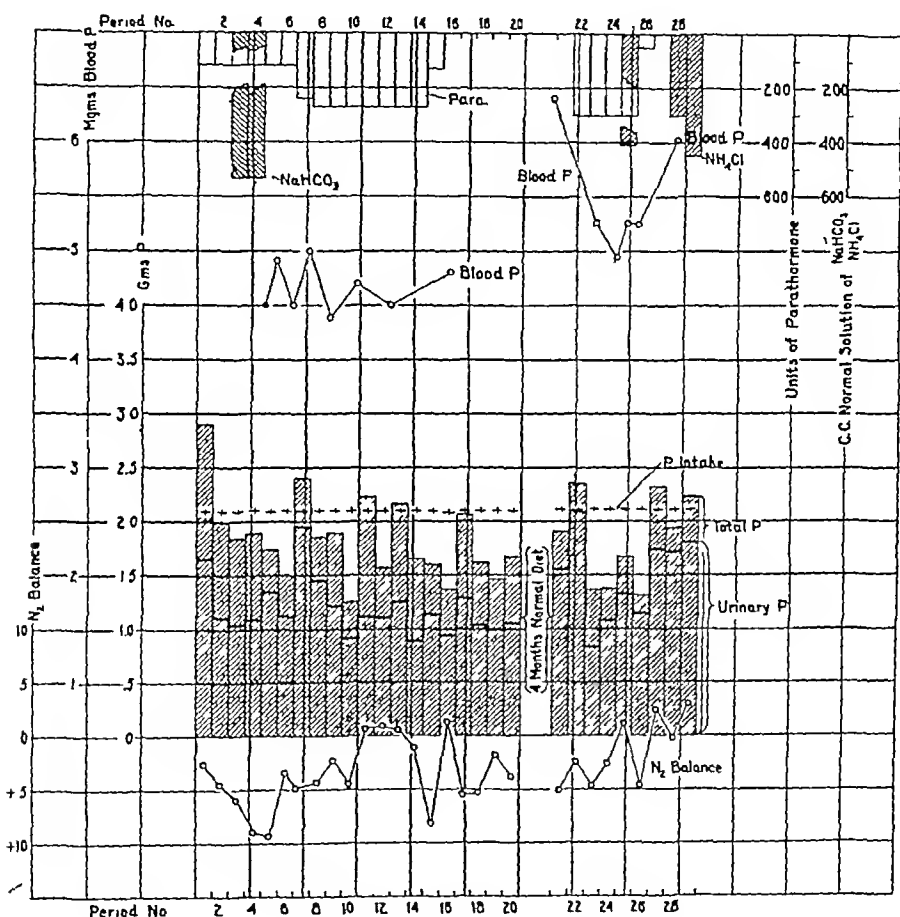


CHART IIIb PHOSPHORUS METABOLISM IN CASE III

sion, however, it is of interest to note the high initial serum phosphorus level, which may be due to the season of the year (7) and to the fact that he was growing (7). As parathormone is administered (period 22), in spite of no effect on the calcium metabolism, there is some rise

in phosphorus excretion, an actual phosphorus excretion in excess of the theoretical phosphorus excretion (chart IIIc), and a corresponding fall of the blood phosphorus. Thus far, if we judge from the phosphorus data, the parathormone effect has been classical. But the

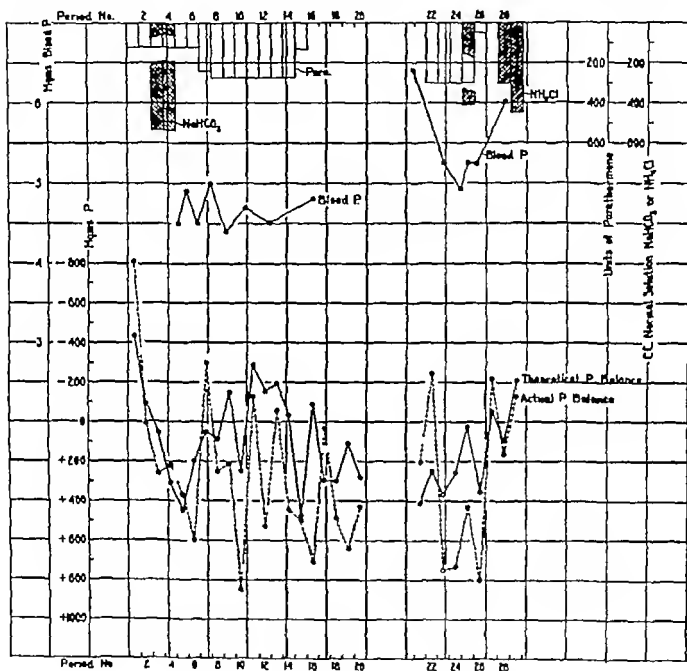


CHART IIIc. "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE III

increase in the actual phosphorus excretion over the theoretical phosphorus excretion in period 22 is not followed by a rise in the calcium excretion. The increased phosphorus excretion lasts only for one period and then falls just as in period 17 of case II (chart IIb). It is of interest that the data of Robinson, Huffman and Burt (25) on the

effect of parathyroid extract on normal calves show one case in which a classical phosphorus response was obtained without any concomitant calcium response. As parathormone is discontinued and ammonium chloride is given, the serum phosphorus again rises to the pre-parathormone level (chart IIIb)

From case III we learn

(xix) In a person who has had parathormone and who has become "immune" to it, the negative calcium balance on a low calcium diet is much reduced or even becomes positive (see also case II)

(xx) The phosphorus response to parathormone administration in a person who fails to show any calcium response is shortlived, but qualitatively normal in this case

(xxi) Sodium bicarbonate administered to this individual did not effect or decrease the effectiveness of parathormone

(xxii) Parathormone administration has not been found helpful in decalcifying an ossifying hematoma (See also case VIII)

*Case IV* The subject of this observation was an Italian laborer of 55 who was suffering from chronic lead poisoning. The effect of parathormone on his lead excretion has been reported as case I by Hunter and Aub (8). We have selected 18 three-day periods on a low calcium diet to report here in more detail. The medication, calcium balance, and blood plasma calcium data are given in chart IV. The nitrogen and phosphorus balances were not determined.

It will be seen that the plasma calcium and the calcium excretion (especially fecal) fall off markedly during the 8 periods (24 days) following parathormone administration. There is then an elevation of the calcium excretion and of the low plasma calcium following ammonium chloride ingestion as shown in the last three periods. The increased calcium excretion appeared largely in the feces.

This experiment confirms observation (ii), that after there has been a marked loss of body calcium produced by parathormone, the negative calcium balance on a low calcium diet is reduced. It also gives us these additional observations

(xxiii) During calcium starvation over long periods of time, especially if there has been an additional loss of calcium due to periods of parathormone administration, the blood calcium tends to fall (see also case III—periods 21-29 on chart IIIa)

(xxiv) A low blood calcium level due to calcium starvation is raised by ammonium chloride (see also case III)

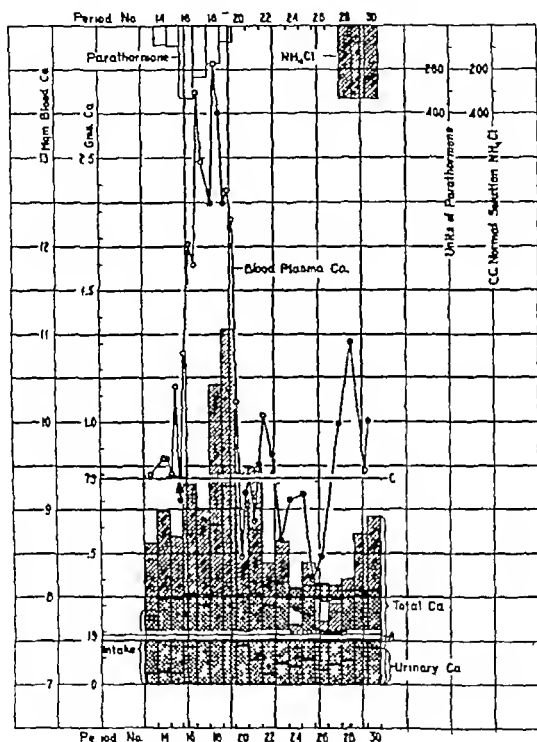


CHART IV CALCIUM METABOLISM IN CASE IV

The blood values in this case are on blood plasma

*Case V* The subject of this investigation was a painter, aged 60, who was suffering from chronic lead poisoning with wrist drop. The results of the studies on lead and calcium excretions during periods

1-36 have been reported by Hunter and Aub (8) as their case IV. We have selected certain of these periods (4-14, 21-23, and 24-36)<sup>4</sup> to report in greater detail and are reporting 19 additional periods on the same patient (5'-24'). The data are shown in charts Va and Vb. It will be noted that periods 4-6 differ from all other periods thus far discussed in that the low calcium in the diet is increased by the addition of calcium lactate.

The most striking part of these data (chart Va) is the extremely high plasma calcium during periods 6, 7, and 8 produced by a dosage of parathormone which was relatively small when compared with that used in previous cases. Furthermore, at these high levels, the urinary calcium excretion does not rise in proportion to the plasma calcium, but remains approximately constant when the plasma calcium rises above 14-15 mgm (cf 20 mgm in period 7). The tendency for the plasma calcium to fall below normal following cessation of parathormone administration and to rise again with ammonium chloride administration is well shown and is confirmatory of observation (xvi) (v supra). Turning to periods 5'-24' one notes that sodium thiosulphate had no effect on either the calcium or the phosphorus metabolism. This does not concern the present subject but is discussed in a paper dealing with the effect of sodium thiosulphate on lead excretion (9). The response to parathormone in periods 10' and 11' is classical. The phosphorus response in the urine is one full period ahead of the calcium response and greater than can be explained by the calcium-phosphorus ratio of bone. The slightly elevated blood serum phosphorus value on the second day of period 11', corresponding to the calcium value of 14.6, is in disagreement with the observation made in cases I-IV, where it was seen that the serum phosphorus falls when the serum calcium rises. This type of reaction is apt to occur when the calcium value has risen over 14 mgm and is associated with a sudden diminution in phosphorus excretion. We believe this is entirely a secondary phenomenon dependent on the high serum calcium. It also accounts for certain sudden falls in the actual phosphorus excretion seen in most of the C charts which do not occur when the calcium is kept at a lower level (cf case VII below). The sodium

<sup>4</sup> The patient left the hospital for one week between periods 23 and 24.

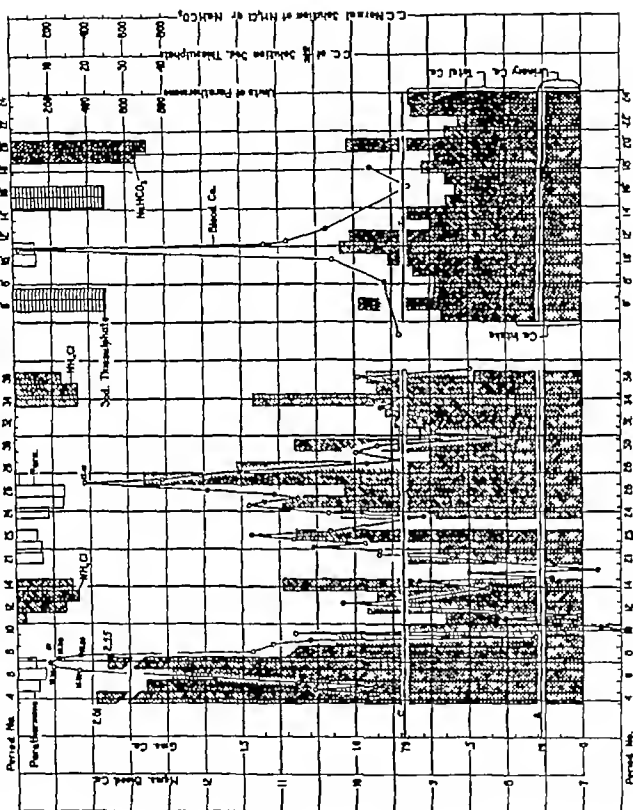


CHART V. CALCIUM METABOLISM IN CASE V

The blood values during the first admission are on blood plasma and during the second admission on blood serum

bicarbonate given in periods 19' and 20' increased both the urinary and fecal calcium and phosphorus, but especially the fecal phosphorus

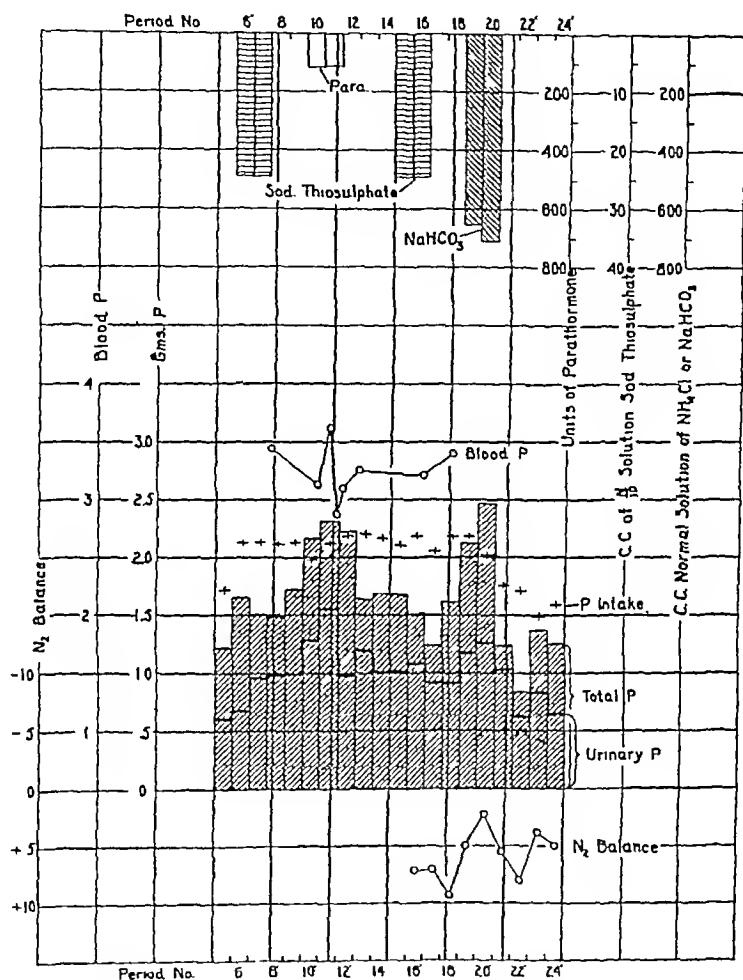


CHART Vb PHOSPHORUS METABOLISM DURING SECOND ADMISSION ON CASE V

(xxv) Parathormone has more effect on the blood calcium level of this patient than on that of any other in our series. Its effect, therefore, may vary in different individuals.

(xxvi) When parathormone is administered to a patient on a rela-

tively high calcium diet, the serum calcium rises more than if the patient were on a low calcium diet

(xxvii) When the serum calcium rises above 14-15 mgm, there is not a corresponding increase in the urinary calcium excretion

(xxviii) When the serum calcium rises above 14-15 mgm, the urinary phosphorus excretion falls and the serum phosphorus rises

(xxix) Sodium bicarbonate when administered alone causes a slight increase in the calcium and phosphorus excretions in the urine and especially in the feces (single observation—see also conclusion (xxi)) We do not differentiate here between excretion by and lack of absorption from the gastro-intestinal tract.

(xxx) Sodium thiosulphate given intravenously is without effect on the calcium and phosphorus excretions in this case.

*Case VI* The subject for this experiment was a college man of 23 who had been afflicted with otosclerosis for ten years. He was studied for 14 periods of 3 days each, and he received parathormone injections for the long consecutive period of 36 days. The data of this experiment are tabulated in charts VIa, VIb and VIc

The phosphorus response to parathormone is unusually gradual but it still appears to be one period ahead of the calcium response. The excess of the actual phosphorus excretion over the theoretical phosphorus excretion is large (periods 5 and 6—chart VIc) and the response in the urinary calcium is correspondingly large. The tremendous increase in the urinary calcium excretion in periods 10 and 11, when ammonium chloride is given in conjunction with parathormone, is most striking. The total is probably beyond the sum of the increased excretions which would have been obtained had the drugs been administered separately. There was no marked change in the serum calcium as a result of the ammonium chloride. But what is perhaps the most important observation from a theoretical point of view is that the increases in the calcium and phosphorus excretions are coincident (chart VIc). The excretion of the extra calcium does not extend into period 12. Thus when calcium phosphate is pulled from the bones by means of ammonium chloride, the calcium and the phosphorus are excreted in the same period. This is suggestive evidence that the excess phosphorus excretion above the theoretical, which is found at the beginning of parathormone administration, is due to an

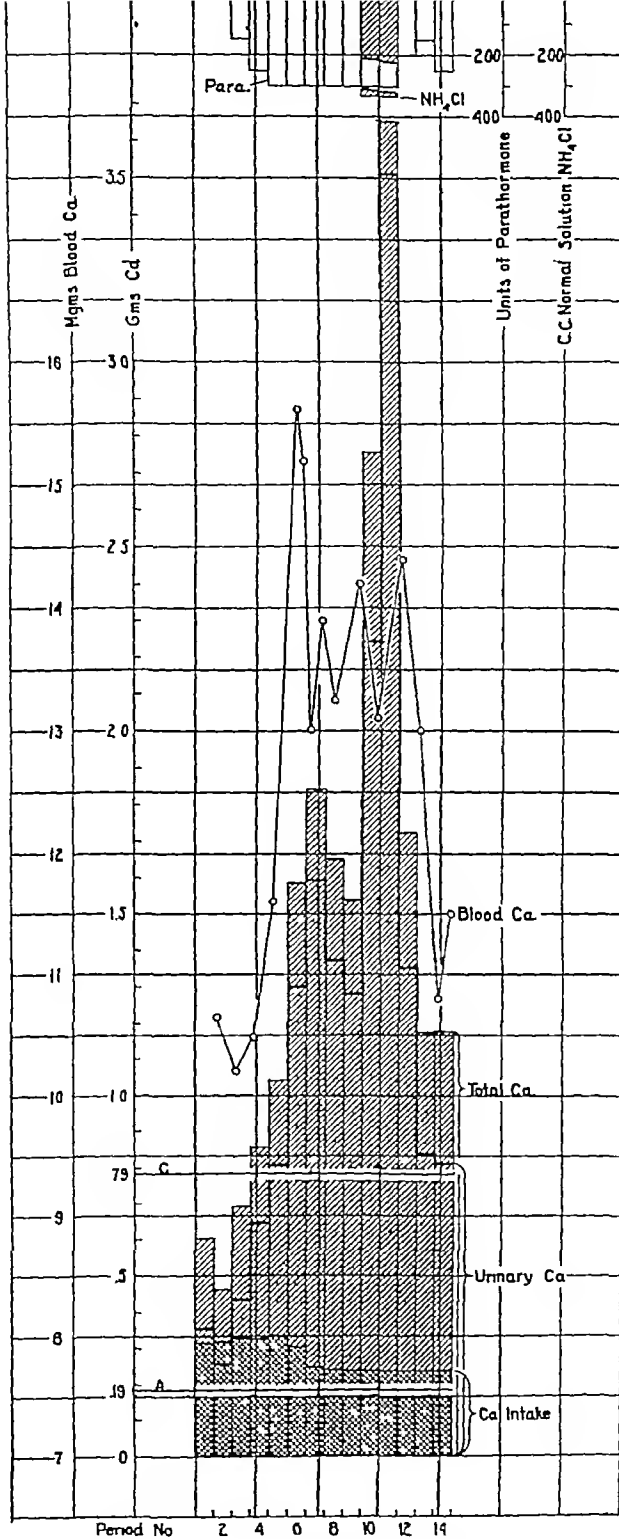


CHART VIa. CALCIUM METABOLISM IN CASE VI

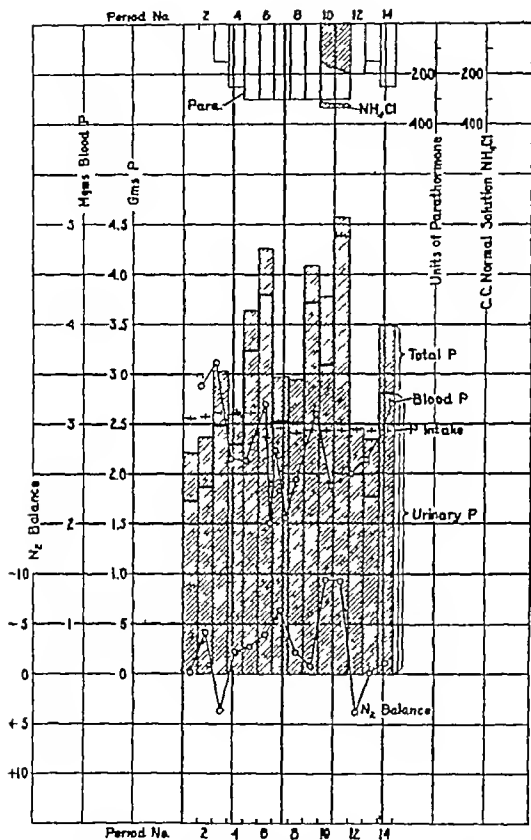


CHART VIIb PHOSPHORUS METABOLISM IN CASE VI

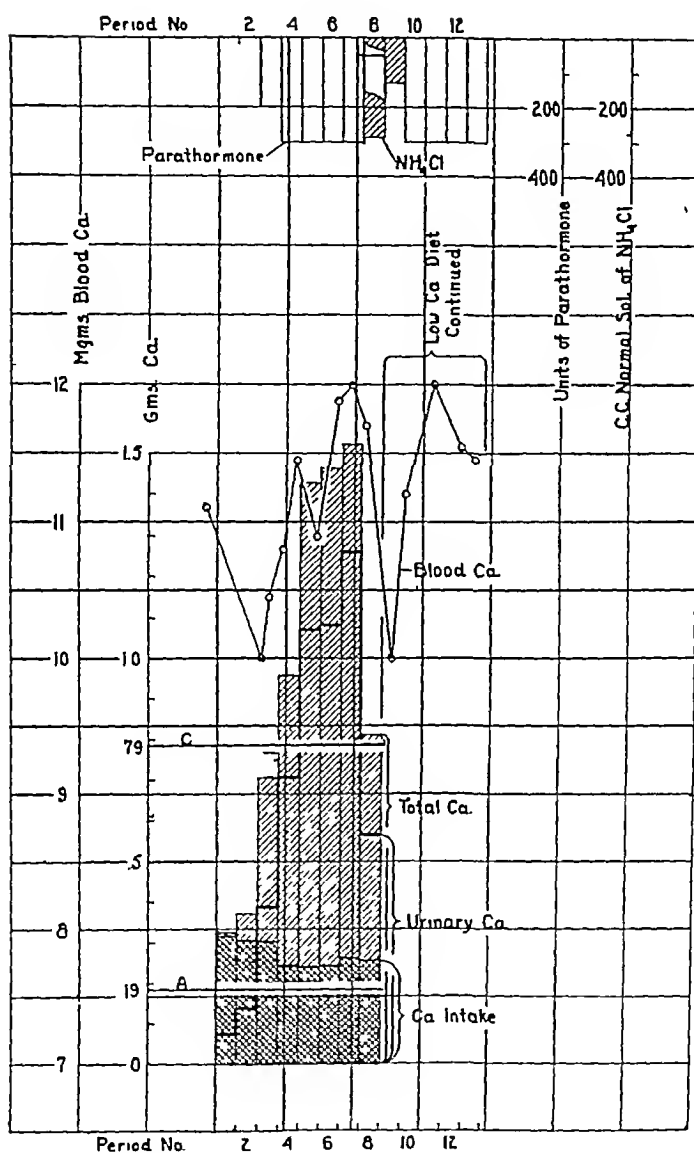


CHART VIIa CALCIUM METABOLISM IN CASE VII

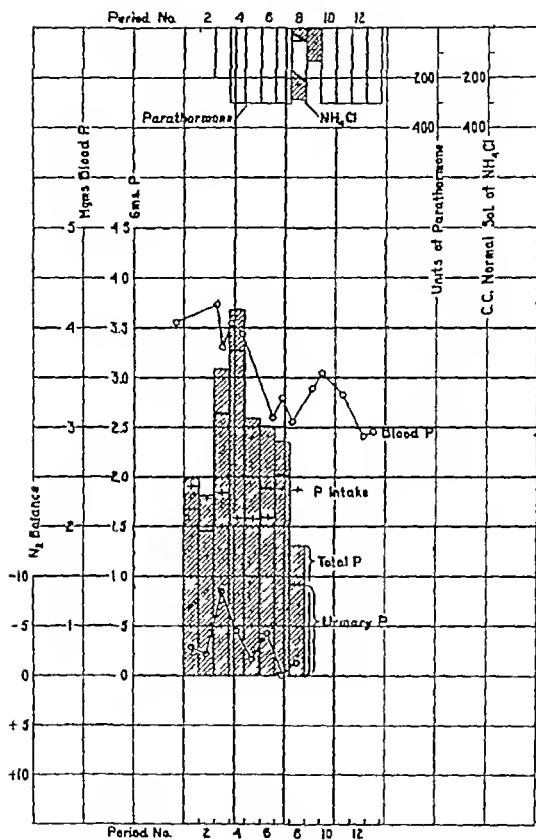


CHART VIII PHOSPHORUS METABOLISM IN CASE VII

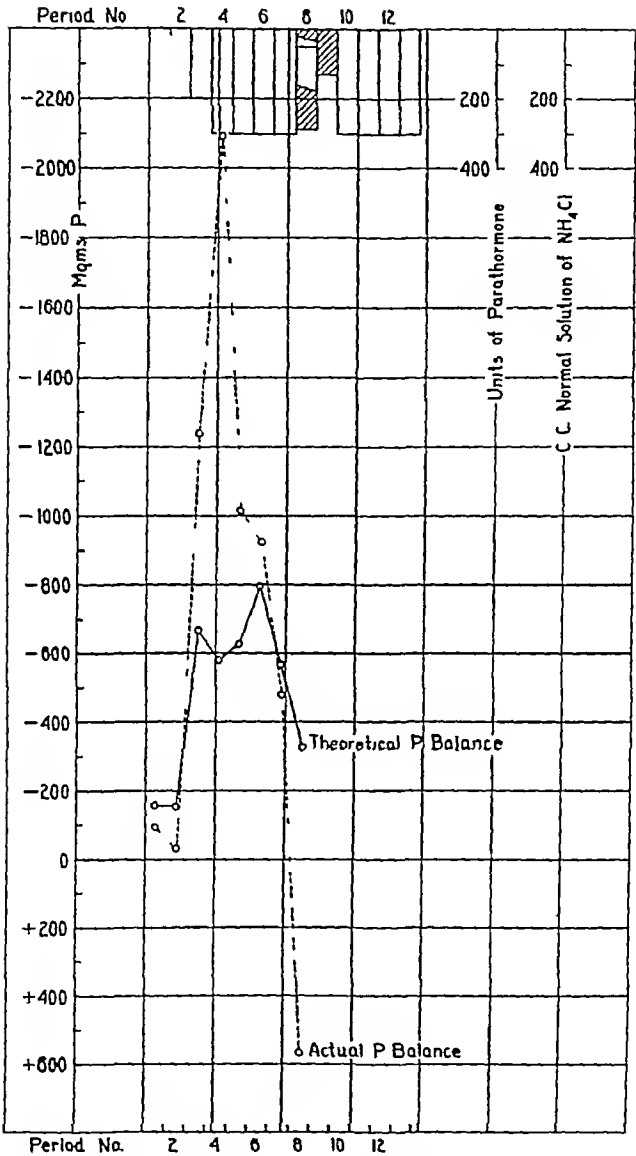


CHART VIIc "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE VII



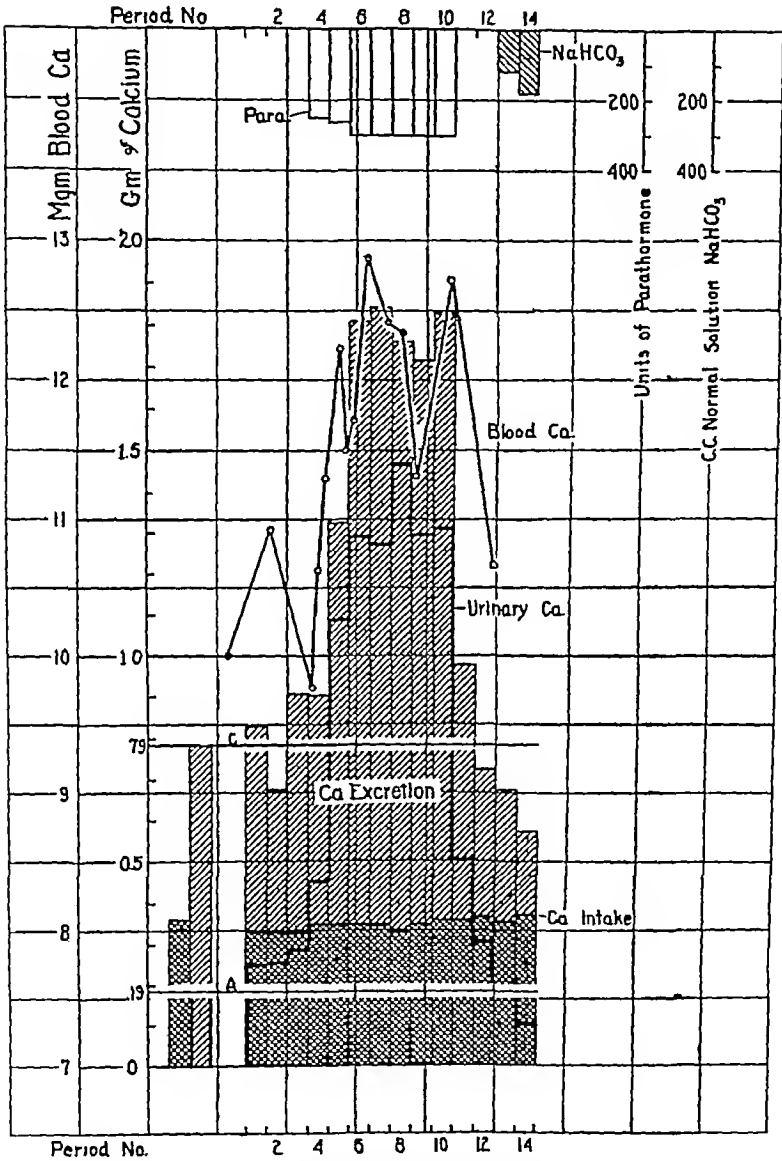


CHART VIIIa CALCIUM METABOLISM IN CASE VIII

Charts VIIIa, VIIIb, and VIIIc correspond to previous charts except that in charts VIIIb and c the actual phosphorus balance was obtained by using the determined phosphorus intake rather than the estimated phosphorus intake. The determined phosphorus intake

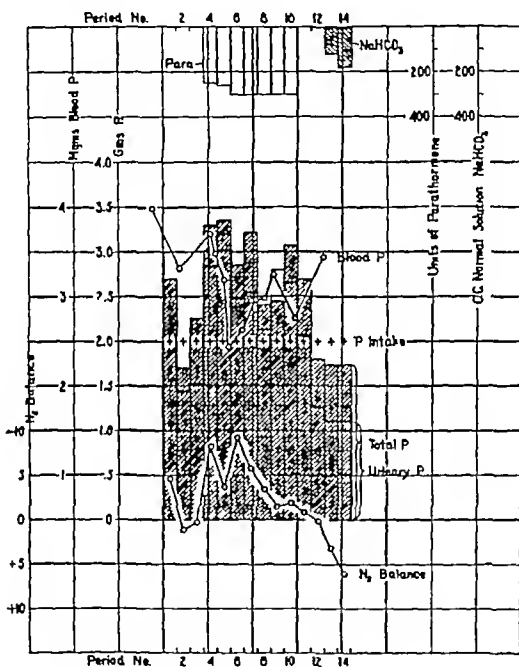


CHART VIIIb PHOSPHORUS METABOLISM IN CASE VIII

per three-day period was 300 mgm lower than the estimated phosphorus intake. This gives an indication of the possible error in the estimation of the phosphorus intake in the previous C-charts, and re-emphasizes the fact that the relative changes in the actual and theoretical phosphorus balances from period to period are more significant

than the absolute differences in any one period. Charts VIIIa, VIIIb, and VIIIc offer further evidence in favor of previous observations but add no new observations. The sodium bicarbonate seems

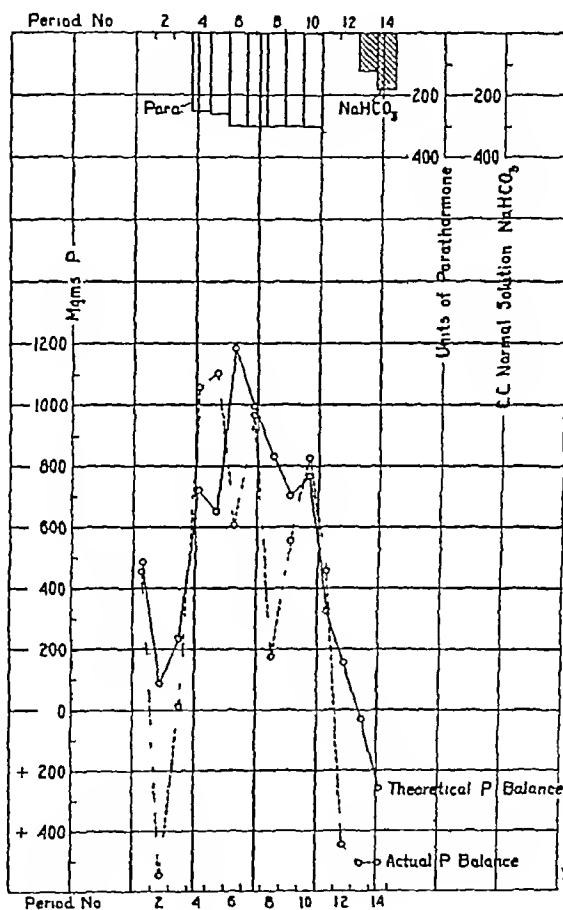


CHART VIIIc "THEORETICAL" AND ACTUAL PHOSPHORUS BALANCES IN CASE VIII

to have had very little effect on the calcium and phosphorus metabolisms (periods 13-17)

In chart VIIIId the effect of parathormone therapy upon the total base balance is recorded. The experiment checks very well in that the total base ingested during the entire experiment is almost identical

with the total base excreted. With parathormone one notes a rise in the urinary excretion of total base, especially periods 3, 4, 8, 9, 10. In the first period following cessation of parathormone injections

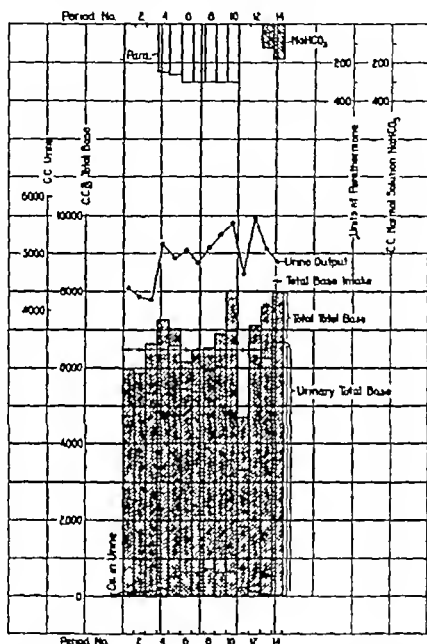


CHART VIII<sup>d</sup> TOTAL BASE METABOLISM IN CASE VIII

All values for base are in cubic centimeter of N/10. This chart also shows the urinary output on a scale chosen such that 1000 cc of urine is equivalent to 1500 cc of N/10 base (cf 1000 cc of body fluid is equivalent to about 1600 cc N/10 base).

there was an extreme drop in the total base excretion which suggests that in this period there was a compensatory retention of total base. But before we can say that this represents an independent action of

parathormone on total base excretion, certain calculations are necessary. It seems fair to assume that the theoretical negative total base balance should equal the total base value of the calcium liberated from the bones plus the total base in the water held by destroyed muscle

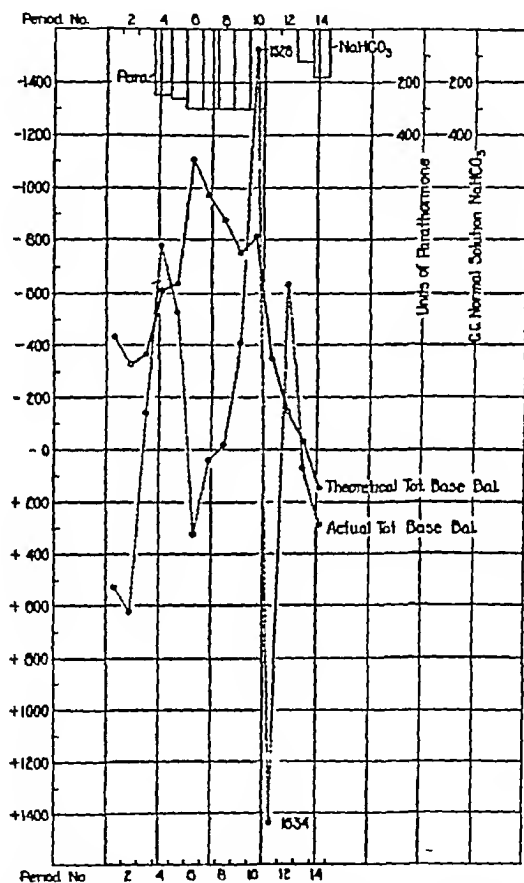


CHART VIIIc "THEORETICAL" AND ACTUAL TOTAL BASE BALANCE IN CASE VIII

(10) Theoretically, any excess excretion of total base beyond this would be at the expense of the total base in the body fluids. We have calculated such a theoretical total base balance and in chart VIIIc this is plotted against the actual total base balance. Here, again, as in the corresponding phosphorus charts, it must be pointed out that

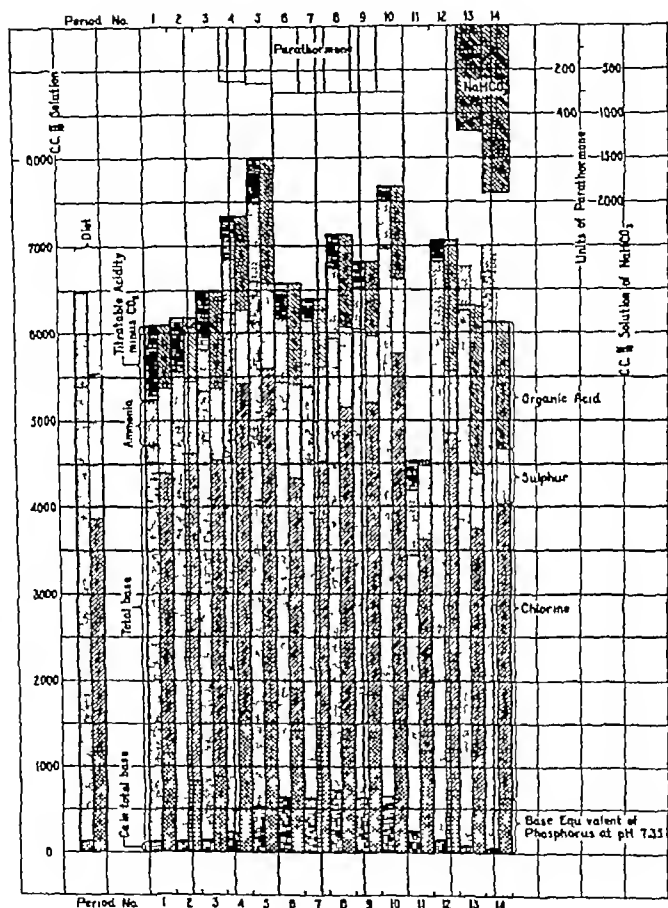


CHART VIII ACID BASE VALUES OF ANIONS AND CATIONS IN URINE IN CASE VIII

Acid-base properties of diet are represented at left. The chlorine and sulphur of the diet have been estimated from tables and so are only approximate. The total base, calcium and phosphorus of the diet were actually determined.

any small percentage error in determining the total base intake makes a large error in the actual base balance, so that the curve representing this may be incorrect by a uniform amount in all periods. In calculating the amount of muscle water liberated or stored from the nitrogen balance the following formula has been used

$$N \times 29.5 \times 0.76 = \text{muscle water (10)}$$

The first factor provides an estimate of the protoplasm destroyed and the second indicates the corresponding water content. The total base content of muscle water has been taken to be 180 cc N/10 per 100 cc (10).

On examination of chart VIIIe, one notes that the curve of the actual total base balance tends to be below the curve of the theoretical total base balance (448 cc N/10 by actual determination) due probably to an error in determining the total base intake. When charts VIId and VIIf are compared, it will be noted that the deviations of the actual from the theoretical excretions coincide fairly well. This leads one to believe that when phosphorus is excreted from the body fluids as a result of parathormone, there is also an excretion of total base held in the body fluids.

Finally chart VIIf has been made. Here the cations of the urine have been balanced against the anions in order to see whether any other anion or cation is affected by parathormone. The principles employed in balancing the electrolytes of the urine are those used by Gamble (10) (11), (12), except that the columns representing the cations and those representing the anions are both shorter by the  $\text{CO}_2$  value of the urine than the corresponding ones of this author. The organic acid value is taken as the difference in the heights of the columns thus established. In periods 13 and 14 the values for the "titratable acidity minus  $\text{CO}_2$ " are negative due to the alkaline urine which resulted from sodium bicarbonate ingestion (12). The values for the intakes are shown on the left of chart VIIf. Calcium, total base, and phosphorus were actually determined in the intake. Sulphur and chlorine were estimated from tables. The chlorine value in the intake is obviously too low, but this is not surprising as the chlorine content of foods varies considerably.

The deductions to be drawn from chart VIIf are mostly negative

ones. The sulphur excretion rises in periods 4, 5, 6, and 7, but so does the negative nitrogen balance. The chlorine varies considerably and tends to follow the total base. This is especially obvious in the fluctuation in periods 9, 10, 11, and 12. Greenwald (13) found a decreased excretion of chloride in the first two days following parathyroidectomy in dogs. The urinary ammonia rose slightly and the titratable acidity fell slightly when parathormone was administered. The organic acid values, which of course contain all the errors of the experiment, show no constant changes.

The chief observation made in this case is a negative one, namely that

(xxxvi) The principle action of parathormone is confined to the calcium and phosphorus metabolisms.

Other observations have not been fully proven but nevertheless are suggested. These are

(xxxvii) During parathormone administration, total base, water and chlorine tend to be excreted from the body fluids coincidentally with the excretion of phosphorus from the body fluids and tend to be retained in the body fluids during the periods of phosphorus retention.

(xxxviii) The sulphur, ammonia and titratable acidity of the urine are little affected by parathormone.

#### DISCUSSION

These observations show quite clearly the metabolic effects of parathormone on the organism. It is an active preparation which has an unequal effect in different individuals. In several of our cases, reported here and in other papers, these effects soon wore off, and there after even large doses had apparently no obvious results. This is an important factor in its prolonged use for medication. The most obvious effect of parathormone, as previously reported by others, is in raising the blood calcium level and thereby increasing the urinary calcium excretion. At first it does this very effectively, but, while it is the only known method for raising appreciably the blood calcium, its effect on the calcium excretion is not as great as that found in thyrotoxicosis (14). It is unfortunate that the effect of parathormone on both calcium and phosphorus metabolism is gradually lost. This

immunity to the drug has been found to remain at least a year in a normal individual and in a patient with tetany

There is evidence among these data that the primary effect of parathormone is on phosphorus excretion rather than on calcium. Thus, when parathormone is administered, the first metabolic changes are a rise in phosphorus excretion and a fall in serum phosphorus, when parathormone is discontinued, the converse is true. At both times the changes in the rate of calcium excretion and in the height of the serum calcium level are more sluggish and lag behind about one period (3 days) (observation (v)). These time relationships are brought out by a study of the "theoretical" and "actual" phosphorus excretions (cf C-charts). The actual phosphorus excretion is in excess of the calculated at the beginning of parathormone administration, and below, following cessation of parathormone. This implies that the extra phosphorus is drawn from the body fluids. It is also interesting that in cases II and III the wearing out of the parathormone effect was manifested first in the phosphorus metabolism. In the second admission of case III (period 21), although parathormone produced no effect on the calcium metabolism, there was a definite but poorly sustained effect on the serum phosphorus and on the phosphorus excretion. This strongly supports the hypothesis that the primary effect of parathormone is on phosphorus.

The above discussion refers only to the primary effect of parathormone on phosphorus metabolism. It was emphasized first by Collip that when a certain critical level of serum calcium has been reached following parathormone injection, the blood phosphorus rises abruptly. This is probably due to an alteration in kidney function resulting from the high serum calcium, for the non-protein nitrogen in the blood also rises. Thus a late effect of parathormone on phosphorus metabolism is brought about, which is exactly opposite to the primary effect.

There is evidence that the relatively greater excretion of phosphorus over calcium following administration of parathormone cannot be attributed to its more ready excretion through the kidney. The situation is not like the sulphur from protein metabolism which is excreted more rapidly than the nitrogen, for in case VI, when a large amount of  $\text{Ca}_3(\text{PO}_4)_2$  was withdrawn from the bones by ammonium chloride, there was no lag in the excretion of the calcium behind that

of the phosphorus Furthermore, when thyroid was given to normal controls (14), the increased excretion of calcium and phosphorus ran fairly parallel

There are several other observations which lend support to the possibility that the primary action of parathormone is on phosphorus Greenwald (13), who has always emphasized the phosphorus changes, has shown that there is a marked retention of phosphorus in the first two days after parathyroidectomy The well known deleterious action of a meat diet on parathyroid tetany (15) (16) may be due to its high phosphorus content (17) Collip (18) showed that following parathyroidectomy in the rabbit the serum phosphorus tended to rise very high without there being much change in the serum calcium, and that parathormone, if administered directly following operation, would prevent this rise In conclusion he stated that, "these observations tend to emphasize the importance of phosphorus in relation to the pathogenesis of tetany" Binger (19) decreased the serum calcium levels in dogs by intravenous injection of phosphates and produced tetany Salvesen, Hastings and McIntosh (20) likewise produced tetany in dogs by feeding huge amounts of phosphates This experiment is the exact antithesis to the report of Palmer and Eckles (21) wherein hypercalcemia, hypophosphatemia and osteomalacia in cows resulted from low phosphorus diets These last two experiments are closely analogous to the situation in hypoparathyroidism and hyperparathyroidism respectively In the first instance, instead of a decreased phosphorus output, there is an increased phosphorus intake, and in the second, instead of an increased phosphorus output there is a decreased phosphorus intake The end results are similar Therefore, the fact that conditions similar to hypoparathyroidism and hyperparathyroidism can be brought about by influencing, through diets, the phosphorus balance, is strong evidence that it is primarily through changes in phosphorus metabolism that the states of hypo- and hyper-parathyroidism are reached

The observation in case VIII (observation (xxxvii)), that there is a tendency to an excretion of base, water, and chloride from the body fluids along with the loss of phosphorus and calcium, is supported by the recent use of parathormone as a diuretic in nephrosis Its re-

ported good effects (22) (23) (24) in this disease may be due to an influence on the excretion of base from the tissue fluids

It is obvious that one effect of parathormone on the calcium-phosphorus metabolism is entirely different from that of acid formers like ammonium chloride or that of the thyroid hormone. Parathormone definitely elevates the blood level of calcium, while the others, which may increase the calcium excretion more than parathormone, do not significantly raise the blood calcium level, unless it is low as in tetany (5)

### CONCLUSIONS

The following may be stated as the main conclusions to be derived from this study of the effect of parathormone on patients maintained on a constant diet containing an inadequate amount of calcium

1 Parathormone administration *gradually* increases the urinary calcium excretion without affecting the fecal excretion. Following cessation of parathormone administration, the urinary calcium excretion *gradually* falls to a level below that found before the administration.

2 The effect on calcium excretion of parathormone and ammonium chloride administered simultaneously is probably more than the sum of their individual effects.

3 The calcium level in the blood is markedly but *gradually* elevated by parathormone injections. The extent of this elevation varies in different individuals, and is more marked when the patient is on a high calcium diet.

4 Parathormone administration *abruptly* increases the urinary phosphorus excretion without affecting the fecal excretion. Following cessation of parathormone administration, the urinary phosphorus excretion *rapidly* falls to a level below that found before the administration. These changes are more rapid than those produced in the calcium metabolism, and greater than can be explained by a theoretical calculation of the phosphorus liberated with calcium and nitrogen.

5 The phosphorus level in the blood is primarily lowered by parathormone. If, however, the serum calcium rises above a critical level of about 14 to 15 mgm, then the urinary phosphorus excretion falls and the blood phosphorus rises.

6 These observations on the effect of parathormone suggest that an increased phosphorus excretion is the primary effect. The excreted phosphorus is partly derived from the body fluids.

7 The effect of parathormone in some cases gradually wears off and the first evidence of this is found in a decreased phosphorus elimination. That this lack of response to parathormone is not due to an exhaustion of calcium reserves, is shown by the fact that ammonium chloride ingestion can still produce an increased elimination in such cases.

8 Nitrogen excretion is not affected by parathormone.

9 Parathormone has not helped to decalcify two ossifying hematomas.

10 The effect of parathormone on other electrolytes is discussed, but no conclusions are formulated.

#### BIBLIOGRAPHY

- 1 Bauer, W, Albright F and Aub, J C, J Clin Invest, 1929, vii, 75. Studies of Calcium and Phosphorus Metabolism. II. The Calcium Excretion of Normal Individuals on a Low Calcium Diet.
- 2 Bauer, W and Aub, J C, J Am Diet Assoc. 1927, iii, 106. Studies of Inorganic Salt Metabolism. I. The Ward Routine and Methods.
- 3 Aub, J C, Albright, F, Bauer W., Salter, W., and Crockett, F., Studies in Calcium and Phosphorus Metabolism. The Calcium Metabolism in Otosclerosis with Observations on the Therapeutic Value of Decalcifying Agents. (To be published.)
- 4 Fitz, R., Alsberg C L., and Henderson, L J., Am J Physiol, 1907, xviii, 113. Concerning the Excretion of Phosphoric Acid During Experimental Acidosis in Rabbits.
- 5 Bauer, W., Albright, F., Rossmesl, E. and Aub, J C., Studies of Calcium and Phosphorus Metabolism. V. The Calcium and Phosphorus Metabolism in Tetany with Observations on the Therapeutic Action of Thyroid and Ammonium Chloride Medication. (To be published.)
- 6 Hamilton, Bengt, Acta Paediatrica, 1922, ii, 1. The Calcium and Phosphorus Metabolism of Prematurely Born Infants.
- 7 Hess, A F., and Lundagen, M A. J Am Med Assoc, 1922, lxxix, 2210. A Seasonal Tide of Blood Phosphates in Infants.
- 8 Hunter, D., and Aub, J C., Quart J Med 1927, xx, 123. Lead Studies. XV. The Effect of the Parathyroid Hormone on the Excretion of Lead and of Calcium in Patients Suffering from Lead Poisoning.

- 9 Ropes, M , and Aub, J C , *Lead Studies The Effect of Sodium Thiosulphate on Lead, Calcium, and Phosphorus Excretion* (To be published )
- 10 Gamble, J L , Ross, G S , and Tisdall, F F , *J Biol Chem* , 1923, lvi, 633 *Metabolism of Fixed Base During Fasting*
- 11 Gamble, J L , and Ross, G S , *Am J Dis Child* , 1923, xxv, 470 *Studies of Tetany II Effect of Ingestion of Hydrochloric Acid Producing Substances on the Acid-base Metabolism of an Infant and the Probable Manner of their Action in the Treatment of Tetany*
- 12 Gamble, J L , *J Biol Chem* , 1922, li, 295 *Carbonic Acid and Bicarbonate in Urine*
- 13 Greenwald, I , and Gross, J , *J Biol Chem* , 1926, lxxviii, 325 *The Effect of Long Continued Administration of Parathyroid Extract upon the Excretion of Phosphorus and Calcium* Greenwald, I , and Gross, J , *J Biol Chem* , 1925, lxxvi, 185 *The Effect of Thyroparathyroidectomy in Dogs Upon the Excretion of Calcium, Phosphorus, and Magnesium* Greenwald, I , *Am J Physiol* , 1911, xxviii, 103 *The Effect of Parathyroidectomy Upon Metabolism I Introduction*
- 14 Aub, J C , Bauer, W , Heath, C , and Ropes, M , *J Clin Invest* , 1929, vii, 97 *Studies of Calcium and Phosphorus Metabolism III The Effect of the Thyroid Hormone and Thyroid Diseases*
- 15 Salvesen, H A , *Acta med Scandinav* , 1923, Supplement 6 *Studies on the Physiology of the Parathyroids*
- 16 Dragstedt, L R , and Peacock, S C , *Am J Physiol* , 1923, lxxiv, 424 *Studies on the Pathogenesis of Tetany I The Control and Cure of Parathyroid Tetany by Diet*
- 17 Greenwald, I , *J Biol Chem* , 1926, lxxvii, 1 *The Effect of the Administration of Calcium Salts and of Sodium Phosphate Upon the Calcium and Phosphorus Metabolism of Thyro-parathyroidectomized Dogs with a Consideration of the Nature of the Calcium Compounds of Blood and their Relation to the Pathogenesis of Tetany*
- 18 Collip, J B , *Medicine*, 1926, v, 1 *The Parathyroid Glands*
- 19 Binger, Carl, *J Pharm and Exper Therap* , 1917, x, 105 *Toxicity of Phosphates in Relation to Blood Calcium and Tetany*
- 20 Salvesen, H A , Hastings, A B , and McIntosh, J F , *J Biol Chem* , 1924, lx, 311 *Blood Changes and Clinical Symptoms Following Oral Administration of Phosphates*
- 21 Palmer, L S , and Eckles, C H , *Proc Soc Exper Biol and Med* , 1927, xxiv, 307 *Effect of Phosphorus Deficient Rations on Blood Composition in Cattle*
- 22 Davidson, J R , *Canad Med Assoc J* , 1925, xv, 803 *A Case of Adolescent Myxedema Accompanied by Nephrosis and by Tetany of Parathyroid Origin Treated with Thyroid and Collip's Parathyroid Extract*

- 23 Mason, E H , Canad Med. Assoc J , 1926, xvi, 538 A Case of Chronic Nephritis Treated with Collip's Parathyroid Extract
- 24 McCann, W S , J Am Med Assoc., 1928, xc, 249 Diuretic Action of Parathyroid Extract—Collip, in Certain Edematous Patients
- 25 Robinson, C S , Huffman, C F , and Burt, K L , J Biol Chem , 1927, lxxvii, 477 The Effect of Parathyroid Extract on Normal Calves



# STUDIES ON THE PHYSIOLOGY OF THE PARATHYROID GLANDS

## I CALCIUM AND PHOSPHORUS STUDIES ON A CASE OF IDIOPATHIC HYPOPARATHYROIDISM

BY FULLER ALBRIGHT AND READ ELLSWORTH

*(From the Medical Clinic of the Johns Hopkins Hospital, Baltimore)*

(Received for publication December 12, 1928)

### INTRODUCTION

In order to determine, if possible, the exact train of events which lead to a rise in the serum calcium following the injection of a potent parathyroid extract, we undertook the present series of investigations. The subject of this study was an Italian boy on whom the diagnosis of idiopathic hypoparathyroidism was made. The criteria on which such a diagnosis can be based will be discussed below.

The injection of a potent parathyroid extract results in four well established changes and any theory as to the action of a parathyroid extract will have to take cognizance of these four cardinal points. They are

- a Rise in serum calcium (1)
- b Rise in urinary calcium excretion (2) (3) (4)
- c Fall in serum phosphorus (5)
- d Rise in urinary phosphorus excretion (2) (3)

Removal of the parathyroid glands results in the converse changes,

i e.,

- a Fall in the serum calcium
- b Fall in the urinary calcium excretion
- c Rise in the serum phosphorus
- d Fall in urinary phosphorus excretion

In addition Albright, Bauer, Ropes, and Aub (3) emphasize the fact that on administration of parathormone, the increase in urinary phosphorus excretion tends to precede the increase in calcium excretion. They further point out that the increased phosphorus appearing in

the urine must come partly from phosphorus dissolved in body fluids and not entirely from the calcium phosphate of the bones. Inasmuch as the calcium and phosphorus changes are probably not simultaneous, it was apparent that, by reducing the metabolism periods to eight hours instead of three days, as in the investigations above (3), we could follow this sequence of events more accurately (cf experiment 1 below). Later we had occasion to reduce the period to 1 hour (cf experiment 2). These shorter periods made it necessary for us to disregard the fecal excretions. This, however, is justifiable, as it has been shown that, following the injection of parathormone, there is a conspicuous alteration in the urinary excretion of calcium and phosphorus, but not in the fecal excretion of these elements (3).

#### REPORT OF CASE

We were fortunate in having the opportunity of studying the effect of parathormone on a patient suffering from idiopathic hypoparathyroidism. The clinical aspects of a case with this rather unique diagnosis are of interest.

The patient, a school-boy of 14 of Italian descent, first entered the Medical Service of the Johns Hopkins Hospital on January 17, 1927 for relief of tetany (J H H No 9974).

The family history was non-contributory.

The past history was uneventful except that he had had measles and chicken pox at 5 years of age.

In 1918, at the age of 5, while under treatment for measles in the Harriet Lane Children's Clinic, it was noted that the patient had markedly hypertrophied tonsils. Tonsillectomy was performed in 1921. Shortly thereafter he began to be troubled at night with attacks of laryngismus. In August, 1922 he had his first attack of carpo-pedal spasm, precipitated by a gastro-intestinal upset. A second attack in January, 1923 was preceded by an upper respiratory infection. Examination at that time at the Harriet Lane Children's Clinic showed carpo-pedal spasm, a bilateral Chvostek sign, and an injected pharynx. The cathodal opening contraction was 10 milliamperes. The serum calcium was 4.9 mgm per 100 cc and the serum phosphorus 10.4 mgm. The blood sodium chloride was 560 mgm per 100 cc.

Ammonium chloride gave him symptomatic relief. However, the general course of the disease was downhill so that at the time of his first admission to the Medical Service he was having each day about fifteen attacks of carpopedal spasm. The individual attack was as a rule precipitated by some unusual exertion such as crossing the street or stealing a base in a ball-game. Momentary loss of consciousness was frequently associated with the attacks.

Physical examination showed a normally developed Italian boy of healthy

appearance. The teeth were in poor repair. Both epitrochlear glands were palpable. Chvostek's and Trousseau's signs were positive.

Other examinations showed a red blood cell count of 5,000,000, hemoglobin of 90 per cent (Sahli), white blood cell count 6600 with a normal differential count, a negative Wassermann reaction, a basal metabolism of minus six, and an entirely normal urine. The plasma calcium was 8.0 mgm per 100 cc. and the phosphorus 10.9 mgm per 100 cc. The whole blood chlorides were 470 mgm. per 100 cc. and the CO<sub>2</sub> combining power 52.4 volumes per cent.

Following the administration of parathormone<sup>1</sup> there was complete relief. The serum calcium was raised to 16.2 mgm. and the serum phosphorus was lowered to 4.7 mgm. Symptoms returned, however, in one week after discontinuing parathormone.

The present, the fourth admission to the Medical Service was on March 21, 1928 for relief for tetany. This time his symptoms had again, as frequently before, been aggravated by a pharyngitis. Examination this time showed in addition to the signs of tetany, a spleen which was just palpable. Ophthalmoscopic examination revealed a posterior, subcapsular, lenticular opacity and several peripheral, radially distributed, lenticular opacities in the right eye. In the left eye a few lenticular opacities were noted. The serum phosphorus was 10.8 mgm per 100 cc and the serum calcium 5.3 mgm per 100 cc. X rays of the bones revealed nothing abnormal.

The features of this case which make it possible to group it with those of hypoparathyroid tetany are

- a. Low serum calcium
- b. High serum phosphorus (in direct contradistinction to the infantile type of tetany where the serum phosphorus is low or normal or only slightly elevated)
- c. Cataracts which so commonly occur in post-operative hypoparathyroidism.
- d. Normal density of bones as shown by X ray (in contradistinction to instances of tetany associated with rickets and osteomalacia)
- e. Aggravation of tetany by exertion. We have found this to be characteristic in several cases of tetany of parathyroid origin.

Thus we believe that, just as myxedema is, as a rule, an idiopathic hypothyroidism, so this case represents an idiopathic hypoparathyroidism.

A report of a similar case is soon to be published from the Massachusetts General Hospital (6). This, together with our case, presents exactly converse findings to those of another patient studied at the Massachusetts General Hospital, in whom a diagnosis of idiopathic hyperparathyroidism was made (7).

#### EXPERIMENT I

*Experimental.* This investigation lasted twenty seven days. An effort was made to have all factors as constant as possible. Conse-

<sup>1</sup> Preparation of parathyroid extract introduced by Collip (1) and supplied by the Eli Lilly Company.

TABLE 1  
Experiment I

| Day | Period | Fluid  |        | Weight | Calcium |                  |                | Phosphorus |                  |                | Serum*         |                | Units of para-thor-mone† | Remarks                     |
|-----|--------|--------|--------|--------|---------|------------------|----------------|------------|------------------|----------------|----------------|----------------|--------------------------|-----------------------------|
|     |        | Intake | Output |        | Intake† | Urine per 100 cc | Total in urine | Intake†    | Urine per 100 cc | Total in urine | Ca             | P              |                          |                             |
|     |        | cc     | cc     | kgm    | mgm     | mgm              | mgm            | mgm        |                  | mgm            | mgm per 100 cc | mgm per 100 cc |                          |                             |
| 1   | 1      | 700    | 240    |        | 195     | 2 3              | 5 5            | 339        | 51 1             | 122            |                |                |                          | Tetany Chvostek             |
|     | 2      | 700    | 575    |        | 195     | 1 0              | 5 9            | 339        | 35 1             | 202            |                |                |                          | Tetany Chvostek             |
|     | 3      | 700    | 395    |        | 195     | 2 6              | 10 1           | 339        | 80 2             | 316            |                |                |                          | Tetany Chvostek             |
| 2   | 4      | 700    | 445    |        | 195     | 1 7              | 7 5            | 339        | 63 7             | 283            |                |                |                          | Tetany Chvostek             |
|     | 5      | 700    | 200    |        | 195     | 2 0              | 3 9            | 339        | 82 9             | 166            | 5 0            | 10 7           |                          | Tetany Chvostek             |
|     | 6      | 700    | 425    |        | 195     | 2 2              | 9 2            | 339        | 64 1             | 271            |                |                |                          | Tetany Chvostek             |
| 3   | 7      | 700    | 540    |        | 195     | 1 4              | 7 7            | 339        | 50 0             | 270            |                |                |                          | Tetany Chvostek             |
|     | 8      | 700    | 940    |        | 195     | 1 7              | 15 6           | 339        | 51 1             | 480            |                |                |                          | Tetany Chvostek             |
|     | 9      | 700    | 430    |        | 195     | 2 8              | 12 2           | 339        | 57 9             | 249            |                |                |                          | Tetany Chvostek             |
| 4   | 10     | 700    | 945    | 45 0   | 195     | 1 5              | 14 6           | 339        | 37 9             | 358            | 5 3            | 10 6           |                          | Tetany Chvostek             |
|     | 11     | 700    | 300    |        | 195     | 2 1              | 6 4            | 339        | 71 9             | 216            | 5 3            | 10 4           |                          | Tetany Chvostek             |
|     | 12     | 700    | 365    |        | 195     | 3 0              | 11 1           | 339        | 81 7             | 298            | 5 2            | 10 9           |                          | Tetany Chvostek             |
| 5   | 13     | 700    | 960    | 44 8   | 195     | 1 0              | 10 0           | 339        | 29 3             | 281            |                |                |                          | Tetany Chvostek             |
|     | 14     | 700    | 465    |        | 195     | 1 4              | 6 6            | 339        | 50 5             | 234            |                |                |                          | Tetany Chvostek             |
|     | 15     | 700    | 590    |        | 195     | 2 3              | 13 8           | 339        | 58 1             | 341            |                |                |                          | Tetany Chvostek             |
| 6   | 16     | 700    | 790    | 44 6   | 195     | 0 6              | 5 1            | 339        | 89 6             | 708            | 5 4            | 10 9           | 50                       | Tetany Chvostek             |
|     | 17     | 700    | 400    |        | 195     | 0 5              | 2 0            | 339        | 104 2            | 417            | 6 2            | 9 5            |                          | No tetany Chvostek negative |
|     | 18     | 700    | 500    |        | 195     | 1 2              | 5 9            | 339        | 91 3             | 456            | 6 7            | 8 8            |                          | No tetany Chvostek negative |

|    |    |     |       |      |     |      |       |     |       |     |      |     |    |           |                   |
|----|----|-----|-------|------|-----|------|-------|-----|-------|-----|------|-----|----|-----------|-------------------|
| 7  | 19 | 700 | 730   |      | 195 | 0 6  | 4 5   | 339 | 92 6  | 675 | 7 1  | 8 4 | 50 | No tetany | Chvostek negative |
|    | 20 | 700 | 540   |      | 195 | 1 9  | 10 0  | 339 | 116 3 | 627 | 8 5  | 7 8 |    | No tetany | Chvostek negative |
|    | 21 | 700 | 510   |      | 195 | 5 7  | 29 1  | 339 | 132 2 | 673 |      | 7 8 |    | No tetany | Chvostek negative |
| 8  | 22 | 700 | 720   | 45 0 | 195 | 3 4  | 24 8  | 339 | 84 6  | 608 | 9 4  | 7 0 | 50 | No tetany | Chvostek negative |
|    | 23 | 700 | 460   |      | 195 | 13 5 | 62 3  | 339 | 122 5 | 564 |      |     |    | No tetany | Chvostek negative |
|    | 24 | 700 | 635   |      | 195 | 14 8 | 94 3  | 339 | 77 9  | 495 | 9 8  | 6 6 |    | No tetany | Chvostek negative |
|    | 25 | 700 | 740   | 44 6 | 195 | 10 0 | 74 0  | 339 | 80 1  | 592 | 9 8  | 6 0 | 50 | No tetany | Chvostek negative |
| 9  | 26 | 700 | 735   |      | 195 | 15 8 | 116 0 | 339 | 88 0  | 589 | 10 5 | 5 4 |    | No tetany | Chvostek negative |
|    | 27 | 700 | 330   |      | 195 | 39 3 | 130 0 | 339 | 119 0 | 392 | 11 2 | 5 7 |    | No tetany | Chvostek negative |
|    | 28 | 700 | 610   | 43 8 | 195 | 8 5  | 51 8  | 339 | 40 2  | 245 | 10 1 | 5 7 |    | No tetany | Chvostek negative |
| 10 | 29 | 700 | 415   |      | 195 | 6 8  | 28 2  | 339 | 43 5  | 181 | 9 3  | 5 9 |    | No tetany | Chvostek negative |
|    | 30 | 700 | 360   |      | 195 | 10 0 | 35 9  | 339 | 37 4  | 135 | 8 8  | 6 5 |    | No tetany | Chvostek negative |
|    | 31 | 700 | 1,090 | 44 1 | 195 | 2 5  | 27 0  | 339 | 22 3  | 121 | 7 8  | 7 0 |    | No tetany | Chvostek negative |
| 11 | 32 | 700 | 270   |      | 195 | 4 4  | 11 9  | 339 | 28 7  | 76  | 7 8  | 7 6 |    | No tetany | Chvostek negative |
|    | 33 | 700 | 470   |      | 195 | 5 9  | 27 6  | 339 | 26 8  | 126 |      |     |    | No tetany | Chvostek negative |
|    | 34 | 700 | 820   | 43 5 | 195 | 3 1  | 25 5  | 339 | 19 2  | 157 |      |     |    | No tetany | Chvostek negative |
| 12 | 35 | 700 | 140   |      | 195 | 4 3  | 6 1   | 339 | 53 1  | 74  | 7 0  | 8 6 |    | No tetany | Chvostek negative |
|    | 36 | 700 | 425   |      | 195 | 11 1 | 48 5  | 339 | 57 3  | 243 |      |     |    | No tetany | Chvostek negative |
|    | 37 | 700 | 1,170 | 43 3 | 195 | 3 9  | 45 5  | 339 | 23 7  | 276 | 7 0  | 8 8 |    | No tetany | Chvostek negative |
| 13 | 38 | 700 | 315   |      | 195 | 7 9  | 24 9  | 339 | 76 0  | 239 |      |     |    | No tetany | Chvostek negative |
|    | 39 | 700 | 615   |      | 195 | 10 7 | 66 0  | 339 | 52 8  | 314 |      |     |    | No tetany | Chvostek negative |

\* Serums for calcium and phosphorus determinations were taken at the beginnings of the periods in which they are tabulated.

† Prepared by Eli Lilly Company following Collip's specification

‡ Actual diets are Appended Diet A was used in periods (1-42), diet B in periods (43-75), diet C in periods (79-81)

TABLE 1  
Experiment I

| Day | Period | Fluid  |        | Weight | Calcium |                         |                      | Phosphorus |                         |                      | Serum*               |                      | Units of para<br>thor<br>monet | Remarks                     |
|-----|--------|--------|--------|--------|---------|-------------------------|----------------------|------------|-------------------------|----------------------|----------------------|----------------------|--------------------------------|-----------------------------|
|     |        | Intake | Output |        | Intake† | Urine<br>per<br>100 cc. | Total<br>in<br>urine | Intake†    | Urine<br>per<br>100 cc. | Total<br>in<br>urine | Ca                   | P                    |                                |                             |
|     |        | cc     | cc     | kgm    | mgm     | mgm                     | mgm                  | mgm        | mgm                     | mgm                  | mgm<br>per<br>100 cc | mgm<br>per 100<br>cc |                                |                             |
| 1   | 1      | 700    | 240    |        | 195     | 2 3                     | 5 5                  | 339        | 51 1                    | 122                  |                      |                      |                                | Tetany Chvostek             |
|     | 2      | 700    | 575    |        | 195     | 1 0                     | 5 9                  | 339        | 35 1                    | 202                  |                      |                      |                                | Tetany Chvostek             |
|     | 3      | 700    | 395    |        | 195     | 2 6                     | 10 1                 | 339        | 80 2                    | 316                  |                      |                      |                                | Tetany Chvostek             |
| 2   | 4      | 700    | 445    |        | 195     | 1 7                     | 7 5                  | 339        | 63 7                    | 283                  |                      |                      |                                | Tetany Chvostek             |
|     | 5      | 700    | 200    |        | 195     | 2 0                     | 3 9                  | 339        | 82 9                    | 166                  | 5 0                  | 10 7                 |                                | Tetany Chvostek             |
|     | 6      | 700    | 425    |        | 195     | 2 2                     | 9 2                  | 339        | 64 1                    | 271                  |                      |                      |                                | Tetany Chvostek             |
| 3   | 7      | 700    | 540    |        | 195     | 1 4                     | 7 7                  | 339        | 50 0                    | 270                  |                      |                      |                                | Tetany Chvostek             |
|     | 8      | 700    | 940    |        | 195     | 1 7                     | 15 6                 | 339        | 51 1                    | 480                  |                      |                      |                                | Tetany Chvostek             |
|     | 9      | 700    | 430    |        | 195     | 2 8                     | 12 2                 | 339        | 57 9                    | 249                  |                      |                      |                                | Tetany Chvostek             |
| 4   | 10     | 700    | 945    | 45 0   | 195     | 1 5                     | 14 6                 | 339        | 37 9                    | 358                  | 5 3                  | 10 6                 |                                | Tetany Chvostek             |
|     | 11     | 700    | 300    |        | 195     | 2 1                     | 6 4                  | 339        | 71 9                    | 216                  | 5 3                  | 10 4                 |                                | Tetany Chvostek             |
|     | 12     | 700    | 365    |        | 195     | 3 0                     | 11 1                 | 339        | 81 7                    | 298                  | 5 2                  | 10 9                 |                                | Tetany Chvostek             |
| 5   | 13     | 700    | 960    | 44 8   | 195     | 1 0                     | 10 0                 | 339        | 29 3                    | 281                  |                      |                      |                                | Tetany Chvostek             |
|     | 14     | 700    | 465    |        | 195     | 1 4                     | 6 6                  | 339        | 50 5                    | 234                  |                      |                      |                                | Tetany Chvostek             |
|     | 15     | 700    | 590    |        | 195     | 2 3                     | 13 8                 | 339        | 58 1                    | 341                  |                      |                      |                                | Tetany Chvostek             |
| 6   | 16     | 700    | 790    | 44 6   | 195     | 0 6                     | 5 1                  | 339        | 89 6                    | 708                  | 5 4                  | 10 9                 | 50                             | Tetany Chvostek             |
|     | 17     | 700    | 400    |        | 195     | 0 5                     | 2 0                  | 339        | 104 2                   | 417                  | 6 2                  | 9 5                  |                                | No tetany Chvostek negative |
|     | 18     | 700    | 500    |        | 195     | 1 2                     | 5 9                  | 339        | 91 3                    | 456                  | 6 7                  | 8 8                  |                                | No tetany Chvostek negative |

|    |    |     |       |      |     |      |      |        |       |     |     |      |           |                   |
|----|----|-----|-------|------|-----|------|------|--------|-------|-----|-----|------|-----------|-------------------|
| 21 | 61 | 800 | 830   | 44 2 | 132 | 5 4  | 44 8 | 187    | 18 9  | 157 | 7 4 | 8 6  | No tetany | Chvostek negative |
|    | 62 | 800 | 440   |      | 132 | 7 5  | 33 0 | 187    | 36 0  | 158 |     |      | No tetany | Chvostek negative |
|    | 63 | 800 | 660   |      | 132 | 10 6 | 70 0 | 187    | 33 1  | 218 |     |      | No tetany | Chvostek negative |
| 22 | 64 | 800 | 630   | 44 1 | 132 | 6 4  | 40 3 | 1 149§ | 25 7  | 164 | 7 4 | 8 7  | No tetany | Chvostek negative |
|    | 65 | 800 | 305   |      | 132 | 7 2  | 21 9 | 1 149  | 118 5 | 363 |     |      | No tetany | Chvostek negative |
|    | 66 | 800 | 575   |      | 132 | 8 9  | 51 1 | 187    | 87 1  | 500 |     |      | No tetany | Chvostek negative |
| 23 | 67 | 800 | 825   | 44 0 | 132 | 3 8  | 31 0 | 668    | 51 8  | 478 | 7 1 | 9 6  | No tetany | Chvostek negative |
|    | 68 | 800 | 625   |      | 132 | 3 2  | 20 0 | 668    | 73 4  | 460 |     |      | No tetany | Chvostek negative |
|    | 69 | 800 | 625   |      | 132 | 6 6  | 41 2 | 668    | 73 6  | 462 |     |      | No tetany | Chvostek negative |
| 24 | 70 | 800 | 970   | 44 0 | 132 | 3 0  | 29 1 | 668    | 53 8  | 522 | 6 9 | 9 5  | No tetany | Chvostek negative |
|    | 71 | 800 | 395   |      | 132 | 3 9  | 15 4 | 668    | 120 8 | 477 |     |      | No tetany | Chvostek negative |
|    | 72 | 800 | 690   |      | 132 | 5 8  | 40 0 | 668    | 72 8  | 495 |     |      | No tetany | Chvostek negative |
| 25 | 73 | 800 | 930   | 44 0 | 132 | 2 8  | 26 0 | 668    | 54 6  | 508 | 6 9 | 10 5 | No tetany | Chvostek negative |
|    | 74 | 800 | 520   |      | 132 | 3 3  | 17 2 | 668    | 91 0  | 490 |     |      | No tetany | Chvostek negative |
|    | 75 | 800 | 590   |      | 132 | 5 8  | 34 2 | 668    | 92 8  | 547 |     |      | No tetany | Chvostek negative |
| 26 | 76 | 800 | 380   | 44 0 | 619 | 4 4  | 16 7 | 1 255  | 128 2 | 487 | 7 0 | 10 3 | No tetany | Chvostek negative |
|    | 77 | 800 | 500   |      | 619 | 4 6  | 23 0 | 1 255  | 105 0 | 525 |     |      | No tetany | Chvostek negative |
|    | 78 | 800 | 470   |      | 619 | 14 6 | 68 7 | 1 255  | 116 7 | 548 |     |      | No tetany | Chvostek negative |
| 27 | 79 | 800 | 1,195 | 44 8 | 480 | 4 8  | 57 4 | 939    | 45 5  | 544 | 7 7 | 9 0  | No tetany | Chvostek negative |
|    | 80 | 800 | 905   |      | 480 | 3 8  | 34 4 | 939    | 75 8  | 686 |     |      | No tetany | Chvostek negative |
|    | 81 | 800 | 510   |      | 480 | 9 4  | 47 9 | 939    | 92 6  | 472 | 7 9 | 8 6  | No tetany | Chvostek negative |

§ Increased phosphorus obtained by adding  $\text{NaH}_2\text{PO}_4$  to diet.

TABLE 1—Continued

| Day | Period | Fluid  |        | Weight | Calcium |                  |                | Phosphorus |                  |                | Serum*         |                | Units of para-thor-mone† | Remarks           |
|-----|--------|--------|--------|--------|---------|------------------|----------------|------------|------------------|----------------|----------------|----------------|--------------------------|-------------------|
|     |        | Intake | Output |        | Intake† | Urine per 100 cc | Total in urine | Intake†    | Urine per 100 cc | Total in urine | Ca             | P              |                          |                   |
|     |        | cc     | cc     | kgm    | mgm     | mgm.             | mgm            | mgm        | mgm              | mgm            | mgm per 100 cc | mgm per 100 cc |                          |                   |
| 14  | 40     | 700    | 760    | 44 0   | 195     | 4 4              | 33 4           | 339        | 33 8             | 257            | 7 1            | 8 7            | No tetany                | Chvostek negative |
|     | 41     | 700    | 420    |        | 195     | 5 8              | 25 0           | 339        | 62 1             | 261            |                |                | No tetany                | Chvostek negative |
|     | 42     | 700    | 615    |        | 195     | 10 9             | 67 0           | 339        | 59 0             | 362            |                |                | No tetany                | Chvostek negative |
| 15  | 43     | 800    | 885    | 43 9   | 132     | 4 9              | 43 5           | 187        | 33 2             | 295            | 7 7            | 8 7            | No tetany                | Chvostek negative |
|     | 44     | 800    | 555    |        | 132     | 5 7              | 31 6           | 187        | 40 0             | 222            |                |                | No tetany                | Chvostek negative |
|     | 45     | 800    | 605    |        | 132     | 10 0             | 60 0           | 187        | 45 9             | 278            |                |                | No tetany                | Chvostek negative |
| 16  | 46     | 800    | 730    | 43 7   | 132     | 4 0              | 29 0           | 187        | 27 2             | 198            | 7 3            | 8 8            | No tetany                | Chvostek negative |
|     | 47     | 800    | 610    |        | 132     | 4 2              | 25 6           | 187        | 38 9             | 237            |                |                | No tetany                | Chvostek negative |
|     | 48     | 800    | 700    |        | 132     | 9 8              | 68 6           | 187        | 30 8             | 216            |                |                | No tetany                | Chvostek negative |
| 17  | 49     | 800    | 705    | 43 3   | 132     | 4 8              | 33 8           | 187        | 20 9             | 147            |                |                | No tetany                | Chvostek negative |
|     | 50     | 800    | 630    |        | 132     | 5 4              | 34 0           | 187        | 27 4             | 173            |                |                | No tetany                | Chvostek negative |
|     | 51     | 800    | 480    |        | 132     | 12 4             | 59 5           | 187        | 43 8             | 211            |                |                | No tetany                | Chvostek negative |
| 18  | 52     | 800    | 830    | 43 7   | 132     | 5 2              | 43 2           | 187        | 22 0             | 182            | 7 7            | 8 6            | No tetany                | Chvostek negative |
|     | 53     | 800    | 560    |        | 132     | 5 5              | 30 8           | 187        | 31 8             | 178            |                |                | No tetany                | Chvostek negative |
|     | 54     | 800    | 595    |        | 132     | 11 9             | 70 8           | 187        | 37 7             | 222            |                |                | No tetany                | Chvostek negative |
| 19  | 55     | 800    | 855    |        | 132     | 5 0              | 42 7           | 187        | 19 0             | 164            |                |                | No tetany                | Chvostek negative |
|     | 56     | 800    | 425    |        | 132     | 6 1              | 25 9           | 187        | 37 8             | 161            |                |                | No tetany                | Chvostek negative |
|     | 57     | 800    | 455    |        | 132     | 12 9             | 58 7           | 187        | 44 2             | 201            |                |                | No tetany                | Chvostek negative |
| 20  | 58     | 800    | 905    | 44 0   | 132     | 4 2              | 37 9           | 187        | 18 2             | 165            | 7 5            | 8 8            | No tetany                | Chvostek negative |
|     | 59     | 800    | 455    |        | 132     | 5 6              | 25 5           | 187        | 34 8             | 158            |                |                | No tetany                | Chvostek negative |
|     | 60     | 800    | 630    |        | 132     | 10 8             | 68 0           | 187        | 33 0             | 211            |                |                | No tetany                | Chvostek negative |

|    |    |     |       |      |     |      |      |       |       |     |     |      |           |                   |
|----|----|-----|-------|------|-----|------|------|-------|-------|-----|-----|------|-----------|-------------------|
| 21 | 61 | 800 | 830   | 44 2 | 132 | 5 4  | 44 8 | 187   | 18 9  | 157 | 7 4 | 8 6  | No tetany | Chvostek negative |
|    | 62 | 800 | 440   |      | 132 | 7 5  | 33 0 | 187   | 36 0  | 158 |     |      | No tetany | Chvostek negative |
|    | 63 | 800 | 660   |      | 132 | 10 6 | 70 0 | 187   | 33 1  | 218 |     |      | No tetany | Chvostek negative |
| 22 | 64 | 800 | 630   | 44 1 | 132 | 6 4  | 40 3 | 1 149 | 25 7  | 164 | 7 4 | 8 7  | No tetany | Chvostek negative |
|    | 65 | 800 | 305   |      | 132 | 7 2  | 21 9 | 1 149 | 118 5 | 363 |     |      | No tetany | Chvostek negative |
|    | 66 | 800 | 575   |      | 132 | 8 9  | 51 1 | 187   | 87 1  | 500 |     |      | No tetany | Chvostek negative |
| 23 | 67 | 800 | 825   | 44 0 | 132 | 3 8  | 31 0 | 668   | 51 8  | 478 | 7 1 | 9 6  | No tetany | Chvostek negative |
|    | 68 | 800 | 625   |      | 132 | 3 2  | 20 0 | 668   | 73 4  | 460 |     |      | No tetany | Chvostek negative |
|    | 69 | 800 | 625   |      | 132 | 6 6  | 41 2 | 668   | 73 6  | 462 |     |      | No tetany | Chvostek negative |
| 24 | 70 | 800 | 970   | 44 0 | 132 | 3 0  | 29 1 | 668   | 53 8  | 522 | 6 9 | 9 5  | No tetany | Chvostek negative |
|    | 71 | 800 | 395   |      | 132 | 3 9  | 15 4 | 668   | 120 8 | 477 |     |      | No tetany | Chvostek negative |
|    | 72 | 800 | 690   |      | 132 | 5 8  | 40 0 | 668   | 72 8  | 495 |     |      | No tetany | Chvostek negative |
| 25 | 73 | 800 | 930   | 44 0 | 132 | 2 8  | 26 0 | 668   | 54 6  | 508 | 6 9 | 10 5 | No tetany | Chvostek negative |
|    | 74 | 800 | 520   |      | 132 | 3 3  | 17 2 | 668   | 91 0  | 490 |     |      | No tetany | Chvostek negative |
|    | 75 | 800 | 590   |      | 132 | 5 8  | 34 2 | 668   | 92 8  | 547 |     |      | No tetany | Chvostek negative |
| 26 | 76 | 800 | 380   | 44 0 | 619 | 4 4  | 16 7 | 1,255 | 128 2 | 487 | 7 0 | 10 3 | No tetany | Chvostek negative |
|    | 77 | 800 | 500   |      | 619 | 4 6  | 23 0 | 1 255 | 105 0 | 525 |     |      | No tetany | Chvostek negative |
|    | 78 | 800 | 470   |      | 619 | 14 6 | 68 7 | 1,255 | 116 7 | 548 |     |      | No tetany | Chvostek negative |
| 27 | 79 | 800 | 1,195 | 44 8 | 480 | 4 8  | 57 4 | 939   | 45 5  | 544 | 7 7 | 9 0  | No tetany | Chvostek negative |
|    | 80 | 800 | 905   |      | 480 | 3 8  | 34 4 | 939   | 75 8  | 686 |     |      | No tetany | Chvostek negative |
|    | 81 | 800 | 510   |      | 480 | 9 4  | 47 9 | 939   | 92 6  | 472 | 7 9 | 8 6  | No tetany | Chvostek negative |

§ Increased phosphorus obtained by adding  $\text{NaH}_2\text{PO}_4$  to diet.

quently the patient was kept in bed and in doors. Each day was divided into three eight-hour metabolism periods. The periods began at 8 a m, 4 p m, and 12 midnight. The patient was given the same meal three times a day at the beginning of each period. Water was likewise administered in equal amounts and at the same relative time in each period. The urine was collected for each period and analyzed for calcium<sup>2</sup> and for phosphorus (9). Vena punctures when done were performed at the beginning of the periods. Fifty units of parathormone were administered at the beginning of periods 16, 19, 22 and 25 (cf. chart 1). The diet was altered at period 43 and several times thereafter as can be noted from table 1. The data for this experiment are given in table 1 and chart 1.

*Results* One notes in control periods 1 to 15 before the administration of parathormone a constantly high serum phosphorus, a correspondingly low serum calcium, and extremely low calcium excretion in the urine, and a phosphorus excretion in the urine comparable with that observed in normal persons on a similar phosphorus intake. This normal urinary phosphorus excretion is not at variance with the four cardinal points mentioned above or with the lowered phosphorus excretion following parathyroidectomy, demonstrated by Greenwald (10). In our case the patient was in equilibrium at a constant level of hypoparathyroidism and was not shifting from a normal to a hypoparathyroid state, as in the post-operative cases. On administration of parathormone the urinary phosphorus excretion rose to its maximum, even exceeding the intake, in the first period (period 16), maintained this high level throughout the period of parathormone administration, and then fell abruptly following cessation of parathormone administration. The serum phosphorus fell coincidentally with the increased phosphorus excretion and rose again when the phosphorus excretion fell off. The serum calcium rose as the serum phosphorus fell and vice versa with almost no tendency for one to lag behind the other. The calcium excretion failed to rise during the five periods following parathormone administration but then rose very abruptly.

The calcium excretion fell off following cessation of parathormone

<sup>2</sup> Fiske method described by Blackfan and Hamilton (8)

administration It should also be noted that the calcium excretion in the urine actually fell slightly during the first five periods after the beginning of parathormone administration Following cessation of

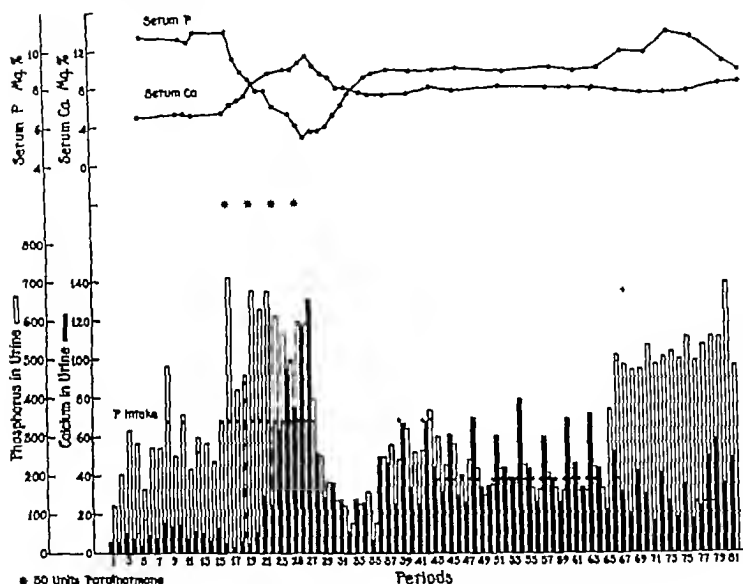


CHART 1 GRAPHIC REPRESENTATION OF DATA IN EXPERIMENT 1

The scale for the serum phosphorus is twice that for the serum calcium. This is so chosen because the normal value for serum calcium is about twice that for serum phosphorus, and because, consequently, a change of 2 mgm of calcium represents the same percentage deviation from normal as a change of one mgm of phosphorus. For convenience in charting, the scale for the urinary calcium excretion is five times that for the urinary phosphorus excretion. Since the ratio of calcium to phosphorus in bone is 2.23 a calcium scale one half that of the phosphorus would have been more logical, although not practical.

parathormone administration the phosphorus excretion fell below the preparathormone level of excretion (cf periods 29 to 35). This is analogous to the decreased phosphorus excretion following parathy-

roidectomy (10) It may be significant that of these four variables, the phosphorus excretion alone seemed to depend quantitatively on the amount of parathormone given The others seemed to depend on how long the parathormone was given and perhaps on how long this high level of phosphorus excretion was maintained

In periods 43 to 63 the phosphorus intake was decreased to a minimum in order to determine whether the serum phosphorus could thus be diminished The result was a decreased phosphorus excretion with practically no change in the serum phosphorus or in the serum calcium Likewise in periods 64 to 75, when the phosphorus intake was greatly increased by the addition of primary sodium phosphate to the diet, the urinary phosphorus excretion was greatly elevated while there was only a slight tendency for the serum phosphorus to rise and for the serum calcium to fall Periods 76 to 81 will not be discussed as the changes in the diet were too radical

*Comment* The observations of experiment I suggest certain conclusions, the interpretation of which may be as follows

1 A state of hypoparathyroidism is associated with a fairly normal urinary phosphorus excretion, a very high serum phosphorus, a low-serum calcium, and a very low urinary calcium excretion

2 Administration of parathormone to an individual in a state of hypoparathyroidism modifies all four of these factors, viz

a The urinary phosphorus excretion immediately rises to its maximum (at least within eight hours)

b Coincidentally the serum phosphorus falls

c The serum calcium rises

d The urinary calcium excretion at first diminishes, and then when the serum calcium is about 8.5, suddenly increases

3 Cessation of administration of parathormone to an individual who had been brought from a hypoparathyroid state to the normal, further modifies these factors, as follows

a The phosphorus excretion at once falls even below the preparathormone level

b The serum phosphorus rises

c The serum calcium falls

d The calcium excretion falls, but less precipitously than the phosphorus excretion

4 Whereas the urinary calcium excretion ordinarily varies with the height of the serum calcium (3), there seems to be a threshold-level at about 8.5 mgm of calcium per 100 cc, below which, the calcium excretion in the urine becomes more or less constant and is independent of the serum calcium value

TABLE 2  
*Experiment II*

| Time        | Fluid intake | Urine     | Calcium in urine | Phosphorus in urine | Chlorine in urine (as NaCl) | Serum                 |                        | Units of parathormone | Remarks  |
|-------------|--------------|-----------|------------------|---------------------|-----------------------------|-----------------------|------------------------|-----------------------|--|
|             |              |           |                  |                     |                             | Calcium               | Phosphorus             |                       |  |
| <i>a.m.</i> | <i>cc</i>    | <i>cc</i> | <i>mgm</i>       | <i>mgm</i>          | <i>grams</i>                | <i>mgm per 100 cc</i> | <i>mgm per 100 cc.</i> |                       |  |
| 7-8         | 250          | 85        | 2.8              | 7.2                 | 0.59                        |                       |                        |                       | Serum CO <sub>2</sub> 51.3 volumes per cent. Serum NaCl 597 mgm. per 100 cc. Refractive in dex 1.3530          |
| 8-9         | 250          | 185       | 1.4              | 6.2                 | 0.50                        | 7.3                   |                        |                       |  |
| 9-10        | 250          | 230       | 1.7              | 6.4                 | 0.69                        |                       |                        |                       |  |
| 10-11       | 250          | 255       | 1.6              | 6.9                 | 0.48                        | 7.5                   | 10.3                   |                       |  |
| 11-12       | 250          | 510       | 3.3              | 39.5                | 1.07                        |                       |                        | 75                    |  |
| <i>p.m.</i> |              |           |                  |                     |                             |                       |                        |                       |  |
| 12-1        | 250          | 270       | 0.9              | 72.0                | 0.32                        | 7.3                   | 10.2                   |                       | Serum CO <sub>2</sub> 52.2 volumes per cent. Serum NaCl 591 mgm. per 100 cc. Refractive in dex of serum 1.3532 |
| 1-2         | 250          | 22        | 1.0              | 62.7                | 0.20                        | 7.5                   |                        |                       |  |
| 2-3         | 250          | 23        | 1.0              | 58.0                | 0.21                        | 7.7                   | 8.8                    |                       |  |
| 3-4         | 250          | 275       | 0.8              | 73.7                | 0.28                        | 8.1                   | 9.3                    |                       |  |
| 4-5         | 250          | 85        | 0.5              | 72.9                | 0.32                        | 8.7                   | 9.2                    |                       |  |
| 5-6         | 250          | 240       | 1.1              | 33.1                | 0.22                        |                       |                        |                       |  |
| 6-7         |              |           |                  |                     |                             | 8.0                   | 9.3                    |                       |  |

\* Blood for Ca and P taken at beginning of period tabulated

5 The high serum phosphorus, present in hypoparathyroidism, does not appear to be altered materially in a week's time either by a very low phosphorus intake or by a very high one

#### EXPERIMENT II

The impression has been prevalent that parathormone is a drug that acts slowly. Collip (1) points out that when parathormone is in-

jected into dogs the peak of the serum calcium curve is reached in twelve to twenty-four hours. In experiment I, we have shown that the maximum effect on urinary phosphorus excretion was reached

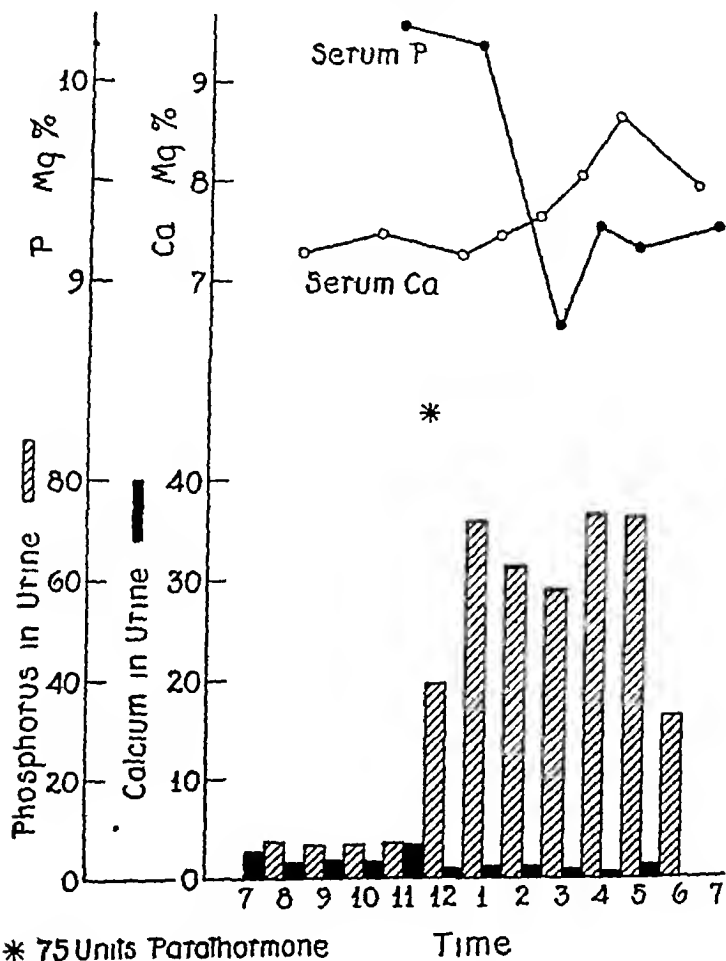


CHART 2 GRAPHIC REPRESENTATION OF CALCIUM AND PHOSPHORUS DATA IN EXPERIMENT 2

The scale for the serum phosphorus is twice that for the serum calcium. The scale for the phosphorus excretion in the urine is one-half that of the calcium excretion.

somewhere during the first eight hours. The second experiment was planned to determine whether this increased phosphorus excretion might occur even earlier than eight hours.

*Experimental* Following a period of 16 days on a low phosphorus diet (0.345 gram per day), the patient fasted and remained in bed for the day of the experiment. He received 250 cc of water at 7 a m and the same amount every hour thereafter until 6 p m when the investigation ceased. The urine was collected every hour and analyzed for calcium, phosphorus and chlorine. At 11 a m 75 units of parathormone were administered. The results can be seen in table 2 and chart 2.

*Results* It will be noted that the excretion of urinary phosphorus increased about 500 per cent in the first hour after parathormone administration and reached its peak by the second hour. In this experiment the serum phosphorus seems to have fallen more quickly than the serum calcium rose. Both of these values had altered within three hours of the injection. The calcium excretion was slightly decreased following the injection (cf experiment I). The increased excretion of water and sodium chloride in the first hour after injection are interesting but will not be discussed here.

*Comment* 6 The action of parathormone on the excretion of urinary phosphorus is maximal within the first hour after injection. It follows from this that future studies must concentrate on the changes occurring in the first hour after injection if one is to learn the fundamental mechanism,—not the remote end results.

### EXPERIMENT III

In the latter part of experiment I it was noted that changes in the amount of phosphorus in the diet had little effect on the serum phosphorus. It was desired to extend this investigation over longer periods of time because of its important theoretical significance.

*Experimental* This experiment started immediately after experiment I and lasted forty days. The time was divided into 10 four day metabolism periods. During the first six periods the patient was on a high phosphorus diet (1.756 gram per day) and during the remaining four on a low phosphorus diet (0.345 gram per day). Except for the change from the high to the low phosphorus diet, the patient received the same food each day. He likewise received the same amount of water each day. He was not kept in bed. The urine for each period was examined for calcium, phosphorus and nitrogen, and the feces

TABLE 3  
Experiment III

| Period | Urine volume |         |       | Calcium |         |                 |                   |             |       |                 |          | Phosphorus                 |         |                 |                   |             |           | Nitrogen        |           |           |             |         | Serum           |         |                           |         |            |  |
|--------|--------------|---------|-------|---------|---------|-----------------|-------------------|-------------|-------|-----------------|----------|----------------------------|---------|-----------------|-------------------|-------------|-----------|-----------------|-----------|-----------|-------------|---------|-----------------|---------|---------------------------|---------|------------|--|
|        | Fluid intake | Day     | Night | Total   | Intake* | Total day urine | Total night urine | Total urine | Feces | Total excretion | Balance  | P equivalent of Ca balance | Intake* | Total day urine | Total night urine | Total urine | Feces     | Total excretion | Balance   | Intake*   | Total urine | Feces   | Total excretion | Balance | P equivalent of N balance | Calcium | Phosphorus |  |
| g      | cc           | cc      | cc    | cc      | gm      | gm              | gm                | gm          | gm    | gm              | gm       | gm                         | gm      | gm              | gm                | gm          | gm        | gm              | gm        | gm        | gm          | gm      | gm              | gm      | gm                        | gm      | gm         |  |
| 1      | 44 1.5 920   |         |       | 5,010.3 | 976     |                 | 0 581.4           | 08.4        | 661   | -0 685          | -0 307.7 | 024                        |         | 5 090.3         | 39                | 8 480       | -1 456.65 | 8.48            | 1         | 6 654.7   | +11.1       | +0 64   | 7 7             | 7 3     |                           | 7 7     | 7 3        |  |
| 2      | 44 5.6 040   | 910.3   | 950.4 | 860.3   | 976     | 0 059           | 0 229             | 0 288.2     | 53.2  | 818             | +1 058   | +0 474.7                   | 024     | 1 010.2         | 960.3             | 970.3       | 69        | 7 660           | -0 636.65 | 8.59      | 2           | 6 665.8 | 0 0             | 0 00    |                           |         |            |  |
| 3      | 44 8.6 040   | 1 020.4 | 125.5 | 145.3   | 976     | 0 041           | 0 177             | 0 218.6     | 16.6  | 378             | -2 402   | -1 077.7                   | 024     | 1 480.3         | 120.4             | 600.3       | 69        | 8 290           | -1 266.65 | 8.50      | 4           | 6 657.0 | +8.8            | +0 51   |                           |         |            |  |
| 4      | 45 1.6 040   | 1 120.2 | 870.3 | 990.3   | 976     | 0 055           | 0 158             | 0 213.3     | 56.3  | 773             | +0 203   | +0 091.7                   | 024     | 1 506.3         | 440.4             | 946.2       | 60        | 7 546           | -0 522.65 | 8.56      | 8           | 6 663.4 | +2.4            | +0 14   |                           |         |            |  |
| 5      | 45 2.6 040   | 1 440.3 | 690.5 | 130.3   | 976     | 0 048           | 0 129             | 0 177.4     | 08.4  | 257             | -0 281   | -0 126.7                   | 024     | 2 190.2         | 790.4             | 980.2       | 70        | 7 680           | -0 656.65 | 8.42      | 0           | 6 648.6 | +17.2           | +0 99   |                           |         |            |  |
| 6      | 45 4.6 040   | 1 150.3 | 650.4 | 800.3   | 976     | 0 024           | 0 113             | 0 137.2     | 32.2  | 457             | +1 519   | +0 681.7                   | 024     | 1 456.3         | 175.4             | 631.2       | 70        | 7 331           | -0 307.65 | 8.52      | 3           | 6 658.9 | +6.9            | +0 40   |                           |         |            |  |
| 7      | 45 5.6 380   | 1 800.3 | 050.4 | 850.5   | 402     | 0 027           | 0 088             | 0 115.4     | 00.4  | 115             | +1 287   | +0 577.1                   | 380     | 0 452           | 0 924             | 1 376.1     | 44        | 2 816           | -1 436.25 | 6.25      | 6           | 2 628.2 | -2.6            | -0 15   |                           |         |            |  |
| 8      | 45 2.6 380   | 2 425.3 | 040.5 | 465.5   | 402     | 0 036           | 0 115             | 0 151.1     | 39.2  | 541             | +2 861   | +1 283.1                   | 380     | 0 235           | 0 453             | 0 688       | 0         | 730             | 1 418     | -0 038.25 | 6.20        | 0       | 2 622.6         | +3.0    | +0 17                     |         |            |  |
| 9      | 45 0.6 380   | 2 625.2 | 920.5 | 545.5   | 402     | 0 039           | 0 117             | 0 156.5     | 24.5  | 396             | +0 006   | +0 002.1                   | 380     | 0 160           | 0 233             | 0 393       | 0         | 730             | 1 123     | +0 257.25 | 6.23        | 8       | 2 626.4         | -0.8    | -0 05                     |         |            |  |
| 10     | 44 8.6 380   | 2 670.3 | 235.5 | 905.5   | 402     | 0 040           | 0 136             | 0 176.4     | 07.4  | 246             | +1 156   | +0 518.1                   | 380     | 0 176           | 0 265             | 0 441       | 0         | 910             | 1 351     | +0 029.25 | 6.19        | 4       | 2 622.0         | +3.6    | +0 21                     |         |            |  |

\* Actual diets appended Diet D used in periods 1-6 diet E in periods 7-10 Ca and P values obtained by analysis of diets

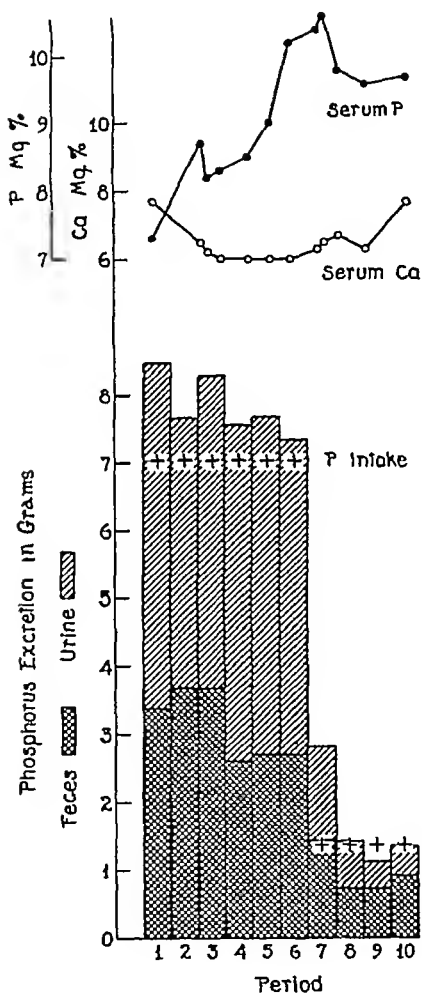


CHART 3 GRAPHIC REPRESENTATION OF PHOSPHORUS METABOLISM IN  
EXPERIMENT 3

for calcium and phosphorus<sup>3</sup> Furthermore, after period 1, because of certain marked discrepancies which were at once noted, the day urine (7 a m to 7 p m) was collected and analyzed separately from the night urine The results are shown in table 3 and chart 3

*Results* Before drawing any conclusions regarding the phosphorus balance, we might have made allowance for the phosphorus deposited with the protein in muscle and with the calcium in bones (11) However, examination of the calcium and nitrogen balances shows that these factors are smaller than the probable errors of the investigation Also, it is true that too much reliance cannot be placed upon balance columns where very high intakes are involved, because a small percentage error in the intake would make a considerable error in the balance Even so, although the serum phosphorus did rise considerably, it is quite apparent from the phosphorus balances (table 3) that, during the first six periods, while on a high phosphorus intake, the patient's kidneys had little difficulty in excreting phosphorus Furthermore, when the phosphorus intake was suddenly lowered the excretion of urinary phosphorus fell immediately rather than slowly, as it would, had there been any previous difficulty in excreting phosphorus

The marked polyuria at night (table 3) was very striking, especially during the first six periods It is also of interest that there was a relative increase in the urinary excretion of calcium, phosphorus, and nitrogen at night proportional to the increased excretion of water Like the polyuria noted in experiment II in the first hour following parathormone injection, these will have to remain as isolated observations for the time being They lead one to suspect some abnormality in the water balance

*Comment* 7 At any given level of hypoparathyroidism there was no marked difficulty in excreting phosphorus nor could the blood chemistry be markedly altered by high and low phosphorus diets

#### DISCUSSION

The serum calcium and serum phosphorus curves in experiment I certainly suggest that one ion rises because the other falls or vice

<sup>3</sup> We are much indebted to Dr Joseph C Aub and his associates at the Massachusetts General Hospital for having the feces analyzed for us

versa One cannot escape the idea that this relationship is dependent on the ability of the serum to hold calcium phosphate, and that, in the presence of normal bones, the blood calcium contains all the calcium and phosphorus ions which its physical state will permit It also seems almost certain that the increased phosphorus excretion, the increased calcium excretion, the decreased serum phosphorus, and the increased serum calcium following parathormone administration are four interrelated facts

Our work, together with that of Albright, Bauer, Ropes, and Aub (3) suggests the following tentative hypothesis as to the *modus operandi*

When parathormone is administered, the equilibria of the body fluids are upset in such a way that an increased phosphorus excretion is a necessary result We do not know the cause of the increased phosphorus excretion, but as a result of this increased phosphorus excretion the body fluids become depleted in phosphorus The falling serum phosphorus is evidence of this As the phosphorus and consequently the phosphate ions in the serum fall, there is a tendency to an unsaturation of the blood with calcium phosphate This tendency is met by a mobilization of calcium phosphate from the bones Thus a deficit in phosphate ions is being supplied by calcium and phosphate ions. Consequently the serum calcium rises With a rise in the serum calcium, provided the level is not below the threshold for calcium excretion, there is a rise in urinary calcium output

The sequence of events which takes place after parathormone administration (chart 1) strongly supports such an hypothesis, except that we have been unable to show consistently that the fall in serum phosphorus precedes, by any detectable interval, the rise in serum calcium

It may be that the calcium phosphorus equilibrium is so finely adjusted that we have not been able to detect the small initial change, or possibly we have not yet studied short enough periods

On the basis of our hypothesis, the question immediately arises as to what causes the increased phosphorus excretion after parathormone injection It seemed at first that there might be a specific impairment of renal function in hypoparathyroidism whereby phosphorus excretion was inhibited However, in experiment III, the

high phosphorus excretion during a high phosphorus diet and the immediate fall to a low phosphorus excretion when the diet was changed to one low in phosphorus would seem to disprove this. All that we can say, therefore, is that some change in the blood equilibria occurs as the result of parathormone administration which makes necessary this increased phosphorus excretion.

#### SUMMARY AND CONCLUSIONS

1 The clinical aspects of a case of idiopathic hypoparathyroidism are reported.

2 The criteria on which the diagnosis of idiopathic hypoparathyroidism is made are

- a* Low serum calcium
- b* High serum phosphorus
- c* Cataract
- d* Normal density of bones by x-ray
- e* Aggravation of tetany by exertion

3 Following the injection of an active parathyroid extract, an increase in phosphorus excretion was immediately detectable and reached its maximum within the first two hours. The mechanism of this is not apparent from our data.

4 Evidence is deduced from study of the metabolism of the patient to show that the increase in serum calcium, the increase in calcium excretion, and the decrease in serum phosphorus following parathormone administration may all be the sequelae of this increase in phosphorus excretion.

5 An hypothesis is suggested to explain these interrelationships.

6 By using high and low phosphorus diets, it seems that there is no inability to excrete phosphorus in hypoparathyroidism and it follows, therefore, that the increased excretion of phosphorus following injection of parathormone is not due to an increase in the excretory ability of the kidney for this element.

7 As the serum calcium rose from 5.2 to 11.2 mgm per 100 cc following the injection of parathormone there was a critical serum calcium value of about 8.5, at which point an almost negligible urinary calcium excretion suddenly changed to a very appreciable one. When the serum calcium was above 8.5 the urinary calcium increased as the

serum calcium rose then decreased abruptly as soon as the serum calcium fell below 8.5 mgm per 100 cc. This suggests that there is a threshold for urinary calcium excretion and that this threshold is below the normal value for serum calcium.

We wish to express our gratitude to Miss M. Struve for her assistance in arranging diets and superintending the collection of specimens.

### BIBLIOGRAPHY

- 1 Collip, J. P. *Medicine* 1926, v, 1. The Parathyroid Glands
- 2 Greenwald, I., and Gross, J. J. *Biol. Chem.* 1926, lxxviii, 325. The Effect of Long Continued Administration of Parathyroid Extract upon the Excretion of Phosphorus and Calcium
- 3 Albright, F., Bauer, W., Ropes, M., and Aub, J. C. *J. Clin. Invest.* 1929, vii, 139. Studies of Calcium and Phosphorus Metabolism. IV. The Effects of the Parathyroid Hormone.
- 4 Hunter, Donald, and Aub, J. C. *Quart. J. Med.*, 1927, xx, 123. Lead Studies. XV. The Effect of the Parathyroid Hormone on the Excretion of Lead and of Calcium in Patients Suffering from Lead Poisoning
- 5 Albright, F., and Bauer, W., The Effect of the Parathyroid Hormone on the Relation of Serum Calcium to Serum Phosphorus. To be published
- 6 Bauer, W., Albright, F., Rossmittel, E., Aub, J. C., Studies of Calcium and Phosphorus Metabolism. The Calcium and Phosphorus Metabolism in Tetany with Observations on the Therapeutic Action of Thyroid and Ammonium Chloride Medication. To be published
- 7 Bauer, W., and Albright, F., Studies of Calcium and Phosphorus Metabolism. A Case of Supposed Hyperparathyroidism with Thyroidectomy. To be published
- 8 Blackfan, K. D., and Hamilton, B., *Johns Hopkins Hosp. Bull.*, 1927, xli, 322. Study of the Inorganic Constituents of Serum in Children with Acute Nephritis
- 9 Benedict, S. R., and Theis, R., *J. Biol. Chem.*, 1924, lxi, 63. A Modification of the Molybdic Method for the Determination of Inorganic Phosphorus in Serum
- 10 Greenwald, I., and Gross, J., *J. Biol. Chem.*, 1925, lxxvi, 185. The Effect of Thyroparathyroidectomy in Dogs upon the Excretion of Calcium, Phosphorus, and Magnesium
- 11 Bauer, W., Albright, F., and Aub, J. C., *J. Clin. Invest.*, 1929, vii, 75. Studies of Calcium and Phosphorus Metabolism. II. The Calcium Excretion of Normal Individuals on a Low Calcium Diet including Data on a Case of Pregnancy



# THE DETERMINATION OF THE CIRCULATING BLOOD VOLUME IN INFANTS BY THE CARBON MONOXIDE METHOD

By RUSTIN McINTOSH

(From the Harriet Lane Home and Department of Pediatrics, the Johns Hopkins University, Baltimore, Maryland)

(Received for publication December 3, 1928)

The carbon monoxide method for determination of blood volume has been used with success in experiments on adults, but has to our knowledge not been previously applied to infants. Miller (1) in 1910 employed the procedure as refined by Zuntz and Plesch (2) in studying a group of thirty children ranging from 6 to 16 years of age, but no reports of studies in younger individuals are available.

The principle of this method was first advocated by Gréhan and Quinquaud (3), and was first applied to the study of blood volume in man by Haldane and Lorrain Smith (4). Improvements in the form of application have been contributed by a number of workers (5), particularly by Salvesen (6) and by Harrop and his associates (7). An accurately measured quantity of carbon monoxide gas is delivered into a breathing apparatus, from which it is absorbed rapidly from the respired air. The time required for maximal absorption, and the conditions under which absorption is accelerated, have been carefully determined by Harrop. After a suitable interval has elapsed for the establishment of equilibrium between the gas in the circulating blood and the respired air, and for complete mixing of the blood, a sample of the latter is withdrawn from a vein and analyzed for carbon monoxide content by an adaptation of the method of Van Slyke and Robschett Robbins (8). When the proper corrections have been applied, the calculation of the circulating blood is then simplified to the following equation:

$$\text{Circulating blood volume} = \frac{\text{Quantity of gas absorbed} \times 100}{\text{Percentage of gas in the blood}} \quad (1)$$

## TECHNIQUE

Pure carbon monoxide is generated from a mixture of formic acid and sulfuric acid with the application of heat, is washed in a trap containing potassium hydroxide solution to remove traces of free acid, and stored in bottles over a mixture of equal parts of glycerine and saturated solution of sodium chloride, a mixture chosen by Harrington and Van Slyke (9) for its low vapor tension and low gas solubility. Samples of the stock of gas are analyzed in the Harrington-Van Slyke

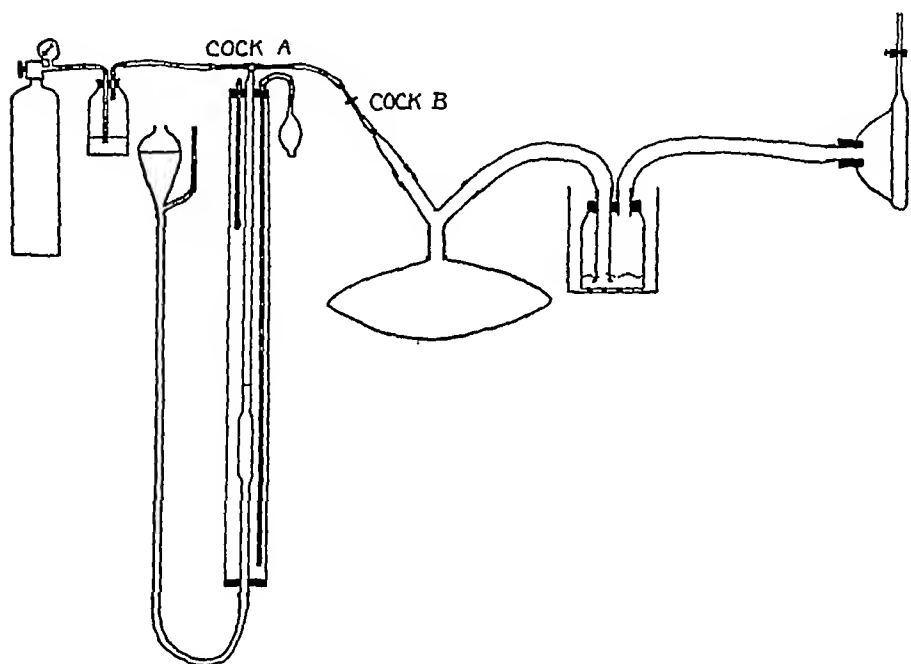


FIG 1 DIAGRAM OF APPARATUS FOR ADMINISTRATION OF A MEASURED AMOUNT OF CARBON MONOXIDE

manometric apparatus to determine the purity factor, the results of parallel determinations agreeing usually within 0.2 per cent. In clinical tests the gas is measured from a specially constructed buret, fitted with a three-way capillary stopcock (cock A in fig 1) at the top, and provided with a water jacket, thermometer, and levelling bulb filled with glycerol-salt mixture, adapted for delivering any quantity of gas up to about 27 cc with an error of measurement of probably less than 0.05 cc.

The breathing apparatus consists of a face-mask, a soda-lime jar, and a rubber balloon, connected by rubber tubing. The face-mask is made of a half-ovoid of transparent celluloid fitted with a rubber pneumatic cushion about the rim and a tight fitting one hole rubber stopper piercing the apex of the dome. The mask is connected to the soda lime jar by a piece of rubber tubing of 17 mm bore. The soda-lime jar is made from a pint mason jar and two test tubes, fixed in place with plaster of paris and sealed with sealing wax. To facilitate changing the soda lime the jar has been broken along a circle about 2 cm from the bottom, so that it may be taken apart, and sealed up with soap and adhesive tape, reinforced with two strong wide rubber bands cut from an inner tube. No. 4 mesh soda-lime is used, held in place by two circular pieces of fine wire gauze, one at the bottom and one at the top of the jar, and is changed after every one or two determinations. During tests on patients the soda lime jar is immersed in a jacket containing ice water to lower the water vapor tension of the rebreathed air and diminish the discomfort of breathing warm air, and to control leakage. A piece of rubber tubing of 17 mm bore connects the soda-lime jar with a large-bore glass Y-tube, to one shank of which is attached a football bladder, and to the other, suitable rubber and glass tubing leading to a one-way stop-cock (cock *B*). All connections are wired or sealed with sealing-wax.

In testing for leaks, the buret was connected to the breathing apparatus by a piece of small bore rubber tubing joining cocks *A* and *B*, and the face-mask was tied firmly against a greased porcelain plate so that the pneumatic cushion was in apposition throughout its circumference. A positive pressure of about 30 cm of glycerol-salt solution was then exerted on the air in the gas buret, and with the levelling bulb clamped in this position the loss of gas pressure was noted. When the leakage amounted to a pressure change of less than 0.15 mm of glycerol salt per minute, the apparatus was considered suitable for use in clinical tests. During actual use, leakage through the division in the soda lime jar is prevented by immersing it in water to a depth of about 10 cm, since the balloon is never allowed to become sufficiently distended, during a test, to put the rubber on the stretch, it is not possible for the air contained in the breathing system to overcome the critical pressure exerted by this protective water-jacket.

Of course, it has not been possible to make control tests of leakage around the edges of the mask when applied to a patient, but this source of error is minimized as far as possible by the liberal application of petrolatum to the pneumatic cushion, by the constant application of firm manual pressure to the mask, and by careful attention to this part of the apparatus throughout the breathing period. In preliminary tests with the mask, which are always made with each patient before carbon monoxide is let into the system, different positions of the mask are tried out until a good apposition is obtained, which can then be held until the end of the experiment.

Before a determination, the gas buret is filled to a convenient level with carbon monoxide and the levelling bulb is clamped in a position causing the contents of the buret to be at a pressure less than atmospheric, at least fifteen minutes are allowed for drainage of solution from the walls of the buret before the pressure is brought to atmospheric and the volume, temperature, and barometric pressure recorded. A small oxygen tank fitted with a water trap and pressure gauge is then connected to the gas buret by one arm of cock *A*. The other arm is connected to cock *B*. The contents of the balloon are expelled, and the tube connecting the face-mask and the soda-lime jar clamped off. With cock *A* turned so as to close the buret but allow communication between the oxygen tank and the breathing apparatus, oxygen is run into the balloon in an amount estimated to be about four or five times the tidal air of the patient. A sample of blood, usually 7 cc to permit duplicate determinations, is now drawn from the patient under paraffin oil and delivered under oil into a bottle containing oxalate. Even in small infants a definite quantity of gas absorbable by Winkler's solution is usually detectable in these preliminary samples, and is probably either evidence of the known contamination of urban atmosphere with carbon monoxide derived from illuminating gas, automobile exhaust gas, and from other sources, or represents a systematic error of the method. The mask, with the pneumatic cushion well greased, is then applied to the patient's face, the clamp removed from the tube connecting the mask with the soda-lime jar, and the patient allowed to breathe into the apparatus for a short period during which the position of the mask is adjusted for the best approximation. With the levelling bulb of the gas buret raised to the

level of cock *A*, and with cock *B* open, carbon monoxide is delivered into the breathing apparatus in an amount estimated to produce a final concentration in the patient's blood of approximately 3 volumes per cent. Cock *A* is then turned so that the buret is closed but the connection between the oxygen tank and the breathing apparatus open, and a slow stream of oxygen allowed to run in at a rate which will keep the balloon nearly empty at full inspiration. A breathing period of ten minutes or more is allowed, at the end of which a second sample of blood is withdrawn under oil while the mask is still in place.

TABLE I  
*Factors by which  $P_{CO}$  is multiplied to calculate volume per cent of CO*

| Temperature | Factor when $P_{CO}$ is measured with gas at 0.5 cc. volume |
|-------------|---|
| °C          |   |
| 20          | 0.02059   |
| 21          | 0.02050   |
| 22          | 0.02042   |
| 23          | 0.02035   |
| 24          | 0.02028   |
| 25          | 0.02021   |
| 26          | 0.02014   |
| 27          | 0.02007   |
| 28          | 0.02000   |
| 29          | 0.01993   |
| 30          | 0.01986   |

In the equation of Van Slyke and Neill,  $\alpha = 0.5$

$S = 15.0$

$i = 1.00$

cc. sample = 3.0

The oxygen stream is then turned off, cock *B* closed, the mask clamped off and removed. The analysis of the blood samples for carbon monoxide is made in duplicate on 3 cc portions by an adaptation of the method of Van Slyke and Robschert-Robbins (8), and the percentage of added carbon monoxide is then determined by subtraction of the average figures. The factors used to convert differential pressure readings to volumes per cent of gas are calculated from the equation of Van Slyke and Neill (10) and are given in table I.

The air resistance in the apparatus so designed is not great, and is

compatible with comfortable rebreathing during periods as long as twenty minutes when tests are made on older children whose confidence can be gained. Most of the infants, naturally, have fretted or cried during the greater part of the breathing period, though a few have slept throughout. Since the rapidity with which the gas is absorbed depends to a large extent on the rate of pulmonary ventilation, it has been customary to prolong the breathing period an additional five or ten minutes in the case of infants who remain quiet. With vigorous crying some of the infants become slightly cyanotic, but not more so than is commonly seen under ordinary circumstances. In a few instances where toxic infants were being studied the color improved as a result of the administration of oxygen incidental to the test. We have observed no harmful effects attributable either to the carbon monoxide specifically or to the test in general, and the disadvantages to the patient are apparently confined to the loss of about 15 cc of blood and the discomfort of the two venapunctures and temporary restriction of activity. The gas does not appear to be retained long, in one patient in whom the test was repeated after 46 hours, the concentration of carbon monoxide had fallen from a level of 3.75 volumes per cent at the termination of the first test to 0.11 volumes per cent at the commencement of the second. Since carboxyhemoglobin does not participate in the oxygen-carrying function of blood, it is obvious that the carbon monoxide method of blood volume determination should not be applied to patients with severe anemia.

#### DISCUSSION OF ERRORS

1 *The denominator in equation I* The analytical error in duplicate determinations by the foregoing technique depends very little on the amount of carbon monoxide present in the sample of blood. In a series of 13 duplicate analyses of samples drawn before any administration of carbon monoxide, the average deviation from the mean in the different pairs of determinations, expressed in volumes per cent of the gas at N.T.P., was  $0.014 \pm 0.012^1$  volumes per cent. In another series of 30 duplicate analyses after carbon monoxide had been given for blood volume determinations, where the final concentration

<sup>1</sup> The second figure indicates the standard deviation

was somewhere between 1.4 and 5.3 volumes per cent, the error expressed in the same way was  $0.025 \pm 0.018$ . Thus, the analytical error may be expressed as follows

$$\text{Analytical Error} = \frac{\text{Sum of absolute errors for both samples} \times 100}{\text{Difference of the mean of the two samples}}$$

$$= \frac{(0.014 \pm 0.012 + 0.025 \pm 0.018) \times 100}{3.00}$$

or approximately 2.3 per cent for an elevation of the carbon monoxide level of 3.00 volumes per cent. For a larger amount of gas administered, the error is correspondingly diminished.

2 *The numerator in equation I* The measurement of the quantity of carbon monoxide absorbed by the patient involves three sources of error: (a) determination of the amount of gas injected into the breathing system, (b) leakage from the system during the breathing period, and (c) determination of the residue of carbon monoxide remaining in the apparatus at the end of the breathing period.

As a matter of convenience precautions are taken to prepare fairly pure carbon monoxide gas by washing out the generator and the storage bottles several times before the final collection of gas for stock. The advantage of making the tests for purity in the Harington-Van Slyke manometric apparatus is twofold: in the first place, satisfactory checks are readily obtained, and in the second place, the use of Winkler's solution for the final absorption of the gas, by an identical procedure to that used in blood analysis, offers a better basis for comparison of results than other methods such as combustion. As was stated previously, the results of duplicate determinations usually agree within 0.2 per cent. The measurement of the gas delivered into the breathing apparatus from the gas buret is held to be accurate to 0.05 cc, including herein the errors associated with barometer and thermometer readings, so that for a volume of 20 cc at room temperature the error from this source amounts to less than 0.25 per cent.

A preliminary test was made to determine the rate of diffusion of carbon monoxide through rubber. Under a positive pressure of about 30 cm. of glycerol salt solution, the pure gas passes through the wall

of an ordinary piece of thin-walled rubber tubing at a rate of approximately 0.0003 cc per square centimeter of surface per hour. At the low partial pressure of CO in the apparatus during a blood volume determination, this leakage does not introduce an appreciable error.

The control of leaks in the breathing apparatus has been dealt with to some extent previously. After every two or three determinations the entire apparatus is tested by immersion in water and exertion of positive pressure on the balloon. It is always possible by the use of sufficient force to cause bubbles to appear around the break in the

TABLE 2  
*Duplicate determinations in individual patients with short time intervals*

| Patient | Date               | Weight | Blood volume | Blood volume | Plasma volume | Plasma volume |
|---------|--------------------|--------|--------------|--------------|---------------|---------------|
|         |                    | kgm    | cc           | cc per kgm   | cc            | cc per kgm    |
| A H     | April 9, 1928      | 7.29   | 620          | 85.0         | 370           | 50.8          |
|         | April 27, 1928     | 7.58   | 560          | 75.2         | 290           | 38.6          |
| M C     | May 17, 1928       | 8.30   | 600          | 72.3         | 337           | 40.6          |
|         | May 19, 1928       | 8.27   | 565          | 68.3         | 323           | 39.1          |
| M A     | September 4, 1928  | 4.72   | 320          | 67.8         | 196           | 41.5          |
|         | September 14, 1928 | 4.81   | 320          | 66.5         | 185           | 38.5          |
| F B     | September 10, 1928 | 4.30   | 270          | 62.8         | 164           | 38.1          |
|         | September 19, 1928 | 4.42   | 300          | 67.9         | 198           | 44.8          |
| L G     | September 15, 1928 | 6.10   | 355          | 58.2         | 218           | 35.7          |
|         | September 26, 1928 | 6.38   | 355          | 55.6         | 199           | 31.2          |

soda-lime jar, but, as was also stated above, this part of the apparatus is sealed off by a water trap during tests on patients. The most obvious source of error in respect to leakage is, of course, the face-mask, to the careful approximation of which the greatest attention is given during the entire breathing period. Experimental control of this factor by duplicate determinations of blood volume on individual patients predicates a basic assumption of the constancy of the blood volume which, in the light of present knowledge, must be considered still an open question. Five such duplicate tests, performed with intervals of

from 2 to 18 days, are listed in table 2 and suggest, at any rate, that the error here is at least not large

The quantity of carbon monoxide absorbed by the patient is the difference between the amount injected into the breathing apparatus and the amount remaining in it at the end of the breathing period. Such residual amounts are small but detectable, and while in our determinations they have not been found to be sufficiently great to influence the final figure for total blood volume, as calculated without taking them into account, by more than 2.7 per cent, and therefore within the total error of the method, it is possible that one may be able at some time to show that in the presence of acidosis they will be large enough to be important, as suggested by the work of Stadie (11) on the absorption coefficient of blood for carbon monoxide under varying hydrogen-ion concentrations. Salvesen (6) in determining the blood volume of rabbits by the carbon monoxide method, had made use of sheep's blood as a reagent for absorbing the residual gas, determining the absorption coefficient of this particular blood by exposing samples of it to varying atmospheric concentrations of carbon monoxide. In our first attempts to utilize this method, with samples of human blood as the absorbing reagent, we were unable to detect the presence of any residual carbon monoxide by simple exposure of the residual air in the apparatus to the blood used. However, since it has been shown that the dissociation curve of carbon monoxide hemoglobin exposed to an atmosphere containing both oxygen and CO is expressed by the equation

$$\frac{\text{HbCO}}{\text{HbO}_2} = K \times \frac{(\text{CO percentage in air})}{(\text{O}_2 \text{ percentage in air})} \quad (\text{II})$$

it seemed likely that this analytical method might be considerably refined by removing the oxygen from the residual air before exposing it to the blood used for absorption of the carbon monoxide.

The method used is similar to that employed by Whipple and his associates (12), except that it requires no special apparatus other than two or three Haldane gas sampling tubes. The volume of residual air is first determined by adding together the contents of the balloon, of the breathing apparatus (exclusive of the balloon) from cock B to the pneumatic cushion of the mask, and of the "dead space" of the

of an ordinary piece of thin-walled rubber tubing at a rate of approximately 0.0003 cc per square centimeter of surface per hour. At the low partial pressure of CO in the apparatus during a blood volume determination, this leakage does not introduce an appreciable error.

The control of leaks in the breathing apparatus has been dealt with to some extent previously. After every two or three determinations the entire apparatus is tested by immersion in water and exertion of positive pressure on the balloon. It is always possible by the use of sufficient force to cause bubbles to appear around the break in the

TABLE 2  
*Duplicate determinations in individual patients with short time intervals*

| Patient | Date               | Weight | Blood volume | Blood volume | Plasma volume | Plasma volume |
|---------|--------------------|--------|--------------|--------------|---------------|---------------|
|         |                    | kgm    | cc.          | cc per kgm   | cc            | cc per kgm    |
| A. H.   | April 9, 1928      | 7.29   | 620          | 85.0         | 370           | 50.8          |
|         | April 27, 1928     | 7.58   | 560          | 75.2         | 290           | 38.6          |
| M. C.   | May 17, 1928       | 8.30   | 600          | 72.3         | 337           | 40.6          |
|         | May 19, 1928       | 8.27   | 565          | 68.3         | 323           | 39.1          |
| M. A.   | September 4, 1928  | 4.72   | 320          | 67.8         | 196           | 41.5          |
|         | September 14, 1928 | 4.81   | 320          | 66.5         | 185           | 38.5          |
| F. B.   | September 10, 1928 | 4.30   | 270          | 62.8         | 164           | 38.1          |
|         | September 19, 1928 | 4.42   | 300          | 67.9         | 198           | 44.8          |
| L. G.   | September 15, 1928 | 6.10   | 355          | 58.2         | 218           | 35.7          |
|         | September 26, 1928 | 6.38   | 355          | 55.6         | 199           | 31.2          |

soda-lime jar, but, as was also stated above, this part of the apparatus is sealed off by a water trap during tests on patients. The most obvious source of error in respect to leakage is, of course, the face-mask, to the careful approximation of which the greatest attention is given during the entire breathing period. Experimental control of this factor by duplicate determinations of blood volume on individual patients predicates a basic assumption of the constancy of the blood volume which, in the light of present knowledge, must be considered still an open question. Five such duplicate tests, performed with intervals of

patient was suffering from tuberculous meningitis, and during the rebreathing period remained on the whole more quiet than the average patient.

In order to compare the results of determinations of blood volume by the carbon monoxide method with those obtained with the dye method, simultaneous tests were performed on ten patients using both methods in the following way. A preliminary control experiment was made to determine whether the addition of brilliant vital red, the dye used, to human blood would interfere with subsequent absorption of carbon monoxide. Ten cubic centimeters of oxalated human blood were placed in each of two beakers of equal size, and to one sample was added 0.05 cc of physiological salt solution, and to the other 0.05 cc

TABLE 5  
*Influence of presence of brilliant vital red in blood on absorption of CO*

| Experiment number | CO content of control blood (saline added) |                  | CO content of test blood (dye added) |                  | Difference (control—dye) |
|-------------------|--|------------------|--------------------------------------|------------------|--------------------------|
|                   | Duplicate analyses                         | Average          | Duplicate analyses                   | Average          |                          |
|                   | volumes per cent                           | volumes per cent | volumes per cent                     | volumes per cent | volumes per cent         |
| 1                 | 1.027<br>1.051                             | 1.04             | 0.924<br>0.923                       | 0.92             | 0.12                     |
| 2                 | 3.776<br>3.742                             | 3.76             | 3.518<br>3.531                       | 3.52             | 0.24                     |

of a 1 per cent solution of the dye in order to give it a final concentration approximately the same as that effected by the use of the dye in clinical tests. Both beakers were then placed in a vacuum desiccator equidistant from the inlet tube, and with the desiccator lid in place, but not tightly sealed, approximately 100 cc of carbon monoxide gas were slowly admitted, displacing some of the contained air. The lid was then sealed and the desiccator gently agitated for 15 minutes to permit the gases to attain equilibrium with the blood samples. On removal of the lid, a layer of paraffin oil, one or two centimeters deep, was added to the contents of each beaker, and the two samples were then analyzed for carbon monoxide content. The results, shown in table 5, agreed within the error of the method, we believe, at any

always in the same direction in that disregarding the correction causes a false high blood volume figure. In comparative determinations on the same patient this is relatively unimportant.

Chang and Harrop (7) found that in the case of adults at rest and breathing quietly, practically complete absorption of the carbon monoxide injected into the breathing apparatus had taken place after 15 minutes of rebreathing. It seemed probable that this interval

TABLE 3  
*Absorption of carbon monoxide as determined by analysis of residual air*

| Patient | CO measured<br>from buret | Unabsorbed CO<br>by analysis | Per cent CO<br>absorbed |
|---------|---------------------------|------------------------------|-------------------------|
|         | cc at N.T.P.              | cc at N.T.P.                 | per cent                |
| S F     | 11 450                    | 0 422                        | 96 31                   |
| J L     | 21 340                    | 0 536                        | 97 49                   |
| D M     | 12 925                    | 0 258                        | 98 00                   |
| M S     | 14 945                    | 0 100                        | 99 34                   |
| L G     | 14 170                    | 0 071                        | 99 50                   |
| R S     | 19 845                    | 0 035                        | 99 82                   |

TABLE 4  
*Control of time required for absorption of CO  
Blood carbon monoxide content*

|     | At start            | After<br>5 minutes  | After<br>10 minutes | After<br>15 minutes |
|-----|---------------------|---------------------|---------------------|---------------------|
|     | volumes<br>per cent | volumes<br>per cent | volumes<br>per cent | volumes<br>per cent |
| A B | 0 077               |                     | 3 43                | 3 30                |
| C L | 0 081               | 2 88                | 2 89                |                     |
| R S | 0 066               | 4 15                | 4 22                |                     |
| L S | 0 077               | 5 12                | 5 27                |                     |

might well be shorter in the case of infants crying vigorously, and the choice of a ten minute breathing period in our technique is based on a small number of control observations which bore out this supposition, and which are given in table 4. This series has not been enlarged on account of the undesirability of removing additional blood, but the results in patient R S, where this control was supplemented by analysis of the residual air for unabsorbed carbon monoxide, show that the ten minute period is, in all probability, adequate. This

patient was suffering from tuberculous meningitis, and during the rebreathing period remained on the whole more quiet than the average patient.

In order to compare the results of determinations of blood volume by the carbon monoxide method with those obtained with the dye method, simultaneous tests were performed on ten patients using both methods in the following way. A preliminary control experiment was made to determine whether the addition of brilliant vital red, the dye used, to human blood would interfere with subsequent absorption of carbon monoxide. Ten cubic centimeters of oxalated human blood were placed in each of two beakers of equal size, and to one sample was added 0.05 cc of physiological salt solution, and to the other 0.05 cc

TABLE 5  
*Influence of presence of brilliant vital red in blood on absorption of CO*

| Experiment number | CO content of control blood (saline added) |                  | CO content of test blood (dye added) |                  | Difference (control—dye) |
|-------------------|--|------------------|--------------------------------------|------------------|--------------------------|
|                   | Duplicate analyses                         | Average          | Duplicate analyses                   | Average          |                          |
| 1                 | volumes per cent                           | volumes per cent | volumes per cent                     | volumes per cent | volumes per cent         |
|                   | 1.027<br>1.051                             | 1.04             | 0.924<br>0.923                       | 0.92             | 0.12                     |
| 2                 | 3.776<br>3.742                             | 3.76             | 3.518<br>3.531                       | 3.52             | 0.24                     |

of a 1 per cent solution of the dye in order to give it a final concentration approximately the same as that effected by the use of the dye in clinical tests. Both beakers were then placed in a vacuum desiccator equidistant from the inlet tube, and with the desiccator lid in place, but not tightly sealed, approximately 100 cc of carbon monoxide gas were slowly admitted, displacing some of the contained air. The lid was then sealed and the desiccator gently agitated for 15 minutes to permit the gases to attain equilibrium with the blood samples. On removal of the lid, a layer of paraffin oil, one or two centimeters deep, was added to the contents of each beaker, and the two samples were then analyzed for carbon monoxide content. The results, shown in table 5, agreed within the error of the method, we believe, at any

rate, the error occurred in a direction which would have given, if anything, an abnormally high blood volume by the carbon monoxide method

In the experiments with patients tabulated in table 6, the dye method of Keith, Rowntree, and Geraghty (13) was used, slightly modified to suit the conditions of work with infants. Either 100

TABLE 6  
*Comparison of CO method and dye method in simultaneous blood volume determinations in 10 patients*

| Patients                 | Weight | Age    | Blood volume<br>(CO method) | Blood volume per kilo-<br>gram (CO method) | Blood volume (dye<br>method) | Blood volume per kilo-<br>gram (dye method) | B V (CO) - B V (dye) | B V (CO) - B V (dye)<br>Weight |
|--------------------------|--------|--------|-----------------------------|--|------------------------------|---|----------------------|--------------------------------|
|                          | kgm    | months | cc                          | cc per kgm                                 | cc                           | cc. per<br>kgm                              | cc                   | cc per<br>kgm                  |
| L S                      | 3 53   | 12     | 275                         | 77 9                                       | 595                          | 168 6                                       | -320                 | -90 7                          |
| F B                      | 4 42   | 2      | 300                         | 67 9                                       | 400                          | 90 5  | -100                 | -22 6                          |
| R W                      | 5 68   | 7      | 445                         | 78 3                                       | 520                          | 91 5  | -75                  | -13 2                          |
| S E                      | 6 72   | 5      | 410                         | 61 0                                       | 720                          | 107 1                                       | -310                 | -46 1                          |
| J L                      | 6 75   | 12     | 500                         | 74 1                                       | 475                          | 70 4  | +25                  | +3 7                           |
| D B                      | 6 87   | 8      | 440                         | 64 1                                       | 500                          | 72 8  | -60                  | -8 7                           |
| W P                      | 6 90   | 15     | 455                         | 66 0                                       | 845                          | 122 5                                       | -390                 | -56 5                          |
| R S                      | 7 47   | 11     | 500                         | 66 9                                       | 550                          | 73 6  | -50                  | -6 7                           |
| C L                      | 7 80   | 17     | 605                         | 77 6                                       | 455                          | 58 3  | +150                 | +19 3                          |
| J J                      | 8 22   | 21     | 655                         | 79 6                                       | 635                          | 77 2  | +20                  | +2 4                           |
| Arithmetical mean        |        |        |                             | 71 $\pm$ 2                                 |                              | 93 $\pm$ 10                                 |                      |                                |
| Standard deviation       |        |        |                             | 6 6 $\pm$ 1 5                              |                              | 31 $\pm$ 7                                  |                      |                                |
| Coefficient of variation |        |        |                             | 9 $\pm$ 2                                  |                              | 33 $\pm$ 7                                  |                      |                                |

1 50 or 2 00 cc of a 1 per cent solution of brilliant vital red, diluted to approximately 10 cc with physiological salt solution, was injected, depending on the size of the patient. The same graduated 2 cc pipet was used to measure the dye to be injected and to make up the standard dilution of dye for use in the colorimeter. For the first sample of blood, 10 cc were removed, of which 4 cc were oxalated under oil

for a single determination of the carbon monoxide content and for hematocrit tests, the rest being allowed to clot in a centrifuge tube for control of the dye method. The dye was then injected, and four or five minutes later the second sample of blood was drawn, 5 or 6 cc in amount, and either oxalated or allowed to clot in a centrifuge tube. A measured amount of carbon monoxide gas was then administered according to the technique outlined previously, and at the end of the usual breathing period 7 or 8 cc of blood were removed and oxalated under oil. In calculating the total blood volume for comparison with the two methods, the figures were corrected by the addition of the amount of blood previously withdrawn. The disagreement between the results of the two methods was often pronounced, and was neither of constant magnitude nor of uniform direction. In seven out of ten such determinations the value of total blood volume expressed in cubic centimeters per kilogram of body weight was higher with the dye method than when the carbon monoxide method was used, and the average of all the determinations with the dye method was 93 cc per kilogram, with the carbon monoxide method, 71 cc per kilogram. The largest discrepancy between the two methods occurred in one patient in whom the blood volume as determined by the dye method exceeded that obtained by the gas method by 320 cc., or 91 cc per kilogram, while, in the other direction, the dye method in another patient gave a result that was lower by 150 cc or 19 cc per kilogram than the value given by the carbon monoxide method. If, as has been suggested by previous workers with both methods, the body weight is in any sense a criterion for the expected blood volume, it is clear from these figures that the carbon monoxide is in our hands the more reliable. Perhaps the obvious conclusion one might wish to draw is that our technique with the dye method is faulty, but we are not convinced that this is a complete explanation of the discrepancy. Whipple and his associates (14) in a comparison of the dye, carbon monoxide, and the exsanguination methods for determining total blood volume in dogs, likewise found discrepancies of similar magnitude and direction. In all but one of fourteen dogs the value of total blood volume expressed in cubic centimeters per kilogram of body weight was higher with the dye method than when the carbon monoxide method was used, and the average of all the determinations

for the dye method was 103.9 cc per kilogram, for the carbon monoxide method, 86.9 cc per kilogram. It is not within the scope of this paper to discuss the theoretical aspects of the application of these different methods, and we do not at the moment contend that the carbon monoxide method as described here eludes all the theoretical pitfalls of clinical determination of the total blood volume, at the same time, it has in our hands exhibited a closer correlation between blood volume and body weight, as expressed in the coefficient of variation, than have determinations made by the dye method, and there is considerable experimental evidence in the literature that the true blood volume follows body weight in health fairly closely, provided suitable allowances are made for conditions of obesity or severe undernutrition.

#### REVIEW OF PREVIOUS WORK IN BLOOD VOLUME OF INFANTS

In this discussion we shall not include the question of the blood volume of newly born infants, since our material does not cover this age group. The investigations of Lucas and Dearing (15) are unique in this field.

The authors just mentioned, in a series of 30 estimations made on 11 nearly normal infants varying in age from two weeks to a year, found an average quantity of 110 cc of blood per kilogram of body weight, as determined by the dye method. Individual patients, though their blood volume might deviate considerably from the average figure when calculated with relation to body weight, nevertheless tended to maintain a fairly constant ratio over a period of as long as two months, which was the maximum interval covered by the duplicate determinations. According to these authors, the ratio of blood volume to body weight is more constant than the ratio plasma volume to body weight. We have calculated the standard deviation and the coefficient of variation<sup>2</sup> of this series and obtain for the latter the figure 9.5.

<sup>2</sup> The formulas for the calculation of these functions are as follows

$m_1$  = a single observation (such as a determination of B V /Wt.)

$n$  = number of observations

$M$  = arithmetical mean of all observations of the type  $m_1$

$$= \frac{\sum m_1}{n}$$

Bakwin and Ravkin (16), also using the dye method, determined the blood volume of 36 normal infants ranging in age from 12 days to 10 months, and obtained an average ratio of 101 cc per kilogram of body weight. In this series the standard deviation was 14.6 and the coefficient of variation 14. Plasma volumes averaged 61 cc per kilogram for the same group, and here the coefficient of variation was 12, indicating a slightly closer correlation than blood volume had shown.

Marrnott and Perkins (17) in a study of 7 approximately normal infants under one year of age obtained with the dye method an average figure of 9.1 per cent of the body weight (which may mean 91 cc per kilogram if their figure refers to volume of blood and not mass), with variations from 8.0 to 10.8 per cent. Their individual determinations were not reported.

Darrow, Soule, and Buckman (18) demonstrated a closer agreement between plasma volume and weight than between blood volume and weight. They showed that for any particular age group a practically identical degree of correlation exists between plasma volume per kilogram and plasma volume per square meter of body surface, and further that the part which age plays is expressed in the fact that the expected plasma volume per kilogram rises from an initial level of 50 cc. per kilogram shortly after birth (calculated from the observations of authors previously quoted) to a level of about 62 cc during the first year of life, subsequently falling slowly to a normal level of 50 cc during the fourth year, where it remains through the rest of childhood, or up to 11 years of age which was the upper limit of their study. They found that the ratio plasma volume to body weight varied more widely during the first year of life than later. The curve of variation with age of the ratio plasma volume per square meter of body surface was more complicated, showing a rounded peak at one year followed by a gradual rise throughout childhood, and in general the ratio blood volume per square meter followed a parallel curve.

$$\text{Standard Deviation} = \sqrt{\frac{\sum (m_i - M)^2}{n}}$$

$$\text{Coefficient of Variation} = \frac{100 \times \text{Standard Deviation}}{M}$$

They calculated the coefficients of variation (referred to by them as the percentage standard deviation) of the ratio plasma volume per kilogram separately for each year of life during the first 11 years and obtained figures ranging from 2 to 8. Such close agreement as these low coefficients express indicates that in average normal children the

TABLE 7  
*Twenty-four blood volume and plasma volume determinations in 22 patients*

| Patient | Blood volume | Plasma volume | Age | Weight | Length | Stem length | Surface area<br>(Du Bois) | Surface area<br>(Meeh) | Surface area<br>(Howland) | Clinical condition for which<br>patient was admitted |
|---------|--------------|---------------|-----|--------|--------|-------------|---------------------------|------------------------|---------------------------|--|
|         | cc           | cc            |     | mos    | kgm    | cm          | cm                        | sq cm                  | sq cm                     |  |
| J G     | 220          | 136           | 1   | 2 18   | 51 5   | 33 5        | 1,740                     | 2,001                  | 1,785                     | Congenital syphilis                                  |
| L S     | 275          | 171           | 12  | 3 53   | 59 5   | 39 5        | 2,375                     | 2,759                  | 2,435                     | Congenital syphilis                                  |
| D M     | 290          | 190           | 2   | 3 55   | 55 5   | 37 5        | 2,265                     | 2,769                  | 2,445                     | Nutritional disturbance                              |
| F B     | 300          | 198           | 2   | 4 42   | 58 0   | 37 0        | 2,567                     | 3,205                  | 2,865                     | Nutritional disturbance                              |
| F T     | 320          | 172           | 5   | 4 58   | 64 0   | 40 5        | 2,800                     | 3,282                  | 2,942                     | Malformation of spine                                |
| M A     | 320          | 185           | 6   | 4 81   | 63 0   | 40 0        | 3,100                     | 3,390                  | 3,053                     | Nutritional disturbance                              |
| J W     | 340          | 225           | 2   | 4 36   | 57 0   |             | 2,520                     | 3,175                  | 2,836                     | Otitis media   |
| E E     | 345          | 209           | 4   | 3 68   | 59 0   | 39 5        | 2,412                     | 2,836                  | 2,507                     | Mongolian idiocy                                     |
| L G     | 355          | 199           | 9   | 6 38   | 70 0   | 43 5        | 3,440                     | 4,093                  | 3,811                     | Pneumonia  |
| C K.    | 370          | 272           | 3   | 3 30   | 54 0   | 35 5        | 2,160                     | 2,638                  | 2,324                     | Prematurity  |
| L H     | 385          | 232           | 5   | 5 20   |        |             |                           | 3,571                  | 3,241                     | Pneumonia  |
| S E     | 410          | 212           | 5   | 6 72   | 72 5   | 40 3        | 3,610                     | 4,238                  | 3,976                     | Nutritional disturbance                              |
| D B     | 440          | 304           | 8   | 6 87   | 65 0   | 40 3        | 3,370                     | 4,300                  | 4,049                     | Pneumonia  |
| R W     | 445          | 315           | 7   | 5 68   | 74 0   | 41 5        | 3,403                     | 3,788                  | 3,474                     | Congenital syphilis                                  |
| W P     | 455          | 263           | 15  | 6 90   | 75 0   | 47 0        | 3,736                     | 4,312                  | 4,062                     | Congenital cataract                                  |
| J L     | 500          | 270           | 12  | 6 75   | 72 0   | 43 0        | 3,589                     | 4,250                  | 3,990                     | Pneumonia, empyema                                   |
| A B     | 505          | 219           | 15  | 7 63   | 75 0   | 49 5        | 3,900                     | 4,611                  | 4,415                     | Nutritional disturbance                              |
| J P     | 535          | 298           | 13  | 6 35   |        |             |                           | 4,080                  | 3,797                     | Pertussis, pneumonia                                 |
| M K.    | 550          | 332           | 9   | 5 73   | 67 5   | 45 0        | 3,190                     | 3,811                  | 3,498                     | Pyuria   |
| A H     | 560          | 291           | 23  | 7 58   | 77 0   |             | 3,966                     | 4,592                  | 4,391                     | Pertussis, encephalitis                              |
| M C     | 565          | 323           | 11  | 8 27   | 73 0   |             | 3,950                     | 4,867                  | 4,724                     | Scarlatina, mastoiditis                              |
| M C     | 600          | 337           | 11  | 8 30   | 73 0   |             | 3,960                     | 4,878                  | 4,739                     | Scarlatina, mastoiditis                              |
| A H.    | 620          | 370           | 22  | 7 29   | 77 0   |             | 3,900                     | 4,473                  | 4,251                     | Pertussis, encephalitis                              |
| J J     | 655          | 367           | 21  | 8 22   | 76 0   | 47 5        | 4,070                     | 4,847                  | 4,700                     | Pertussis, pneumonia                                 |

body weight is more than a fair index of the total plasma volume as determined by the dye method. It is further suggested by these authors that plasma volume appears to be related primarily to metabolic rate, and that its relations to body weight and surface area are of secondary degree and subject to the same limitations as govern the

estimation of metabolic rate from measurements of body mass and area

#### EXPERIMENTAL RESULTS

Table 7 presents the results of 24 determinations of blood volume in 22 hospital patients admitted for a variety of causes and who, at the time the determination was made, were not thought to be suffering from any circumstance that would bring about a temporary alteration of blood volume. In other words, the figure obtained for each patient was considered to represent a normal level for that individual at that particular time. Only the age group of the first two years is included. As a measure of the normal blood volume at this age they, of course, suffer the defect of not comprising any strictly normal individuals, yet conversely, by including a variety of nutritional states they are possibly better adapted for showing what correlations are most tenaciously held when the individual deviates widely from the average, and may thus serve to demonstrate, for example, whether body weight or surface area is a measure of total blood volume, even better than would an assembly of entirely normal values.

In comparison of our results with those obtained by others with the dye method, the fundamental differences between the two methods must be borne in mind: first, that the dye method measures the plasma volume directly while the carbon monoxide method measures the circulating blood volume directly, and secondly, that in the former method the added substance is mixed entirely or mainly in the plasma, whereas in the latter the gas is taken up almost exclusively by the red cells. According to Whipple and his associates (14), these differences are largely responsible for the higher figures which so many observers have obtained in working with the dye method, and in their hands the carbon monoxide method has given in dogs a higher figure than is yielded by the exsanguination method, though it closely approximates the results of the bleeding method where the latter is combined with procedures adapted for including in the total blood volume a figure calculated from the hemoglobin derived from extraction of tissues—in other words, when the non-circulating hemoglobin is included in the total blood volume figure. We do not agree with the statement made by Darrow, Soule, and Buckman (18) that the carbon monoxide method gives results that are lower than the actual blood volume.

In table 7 we have listed the determined values for blood volume and plasma volume, together with the age, body weight, length, stem length, and body surface as calculated by the three different formulae of Du Bois, Meeh, and Howland (19). The coefficients of correlation (20) in table 8 express the degree of agreement between observations of blood volume or plasma volume and the corresponding measurement of the function under consideration, in the sense that no agreement at all is indicated by a coefficient of 0, perfect agreement by a coefficient of  $\pm 1$ . For a more detailed discussion of the significance of coefficients of correlation and variation, one is referred to appropriate

TABLE 8  
*Coefficients of correlation of blood and plasma volume with various body measurements*

|  | Blood volume     | Plasma volume   |
|--|------------------|-----------------|
| Age  | 0.79 $\pm$ 0.08* | 0.63 $\pm$ 0.12 |
| Body weight                                | 0.89 $\pm$ 0.05  | 0.72 $\pm$ 0.11 |
| Body length                                | 0.86 $\pm$ 0.06  | 0.68 $\pm$ 0.11 |
| (Body length) <sup>2</sup>                 | 0.86 $\pm$ 0.06  | 0.68 $\pm$ 0.11 |
| (Body length) <sup>3</sup>                 | 0.86 $\pm$ 0.06  | 0.68 $\pm$ 0.11 |
| Stem length                                | 0.73 $\pm$ 0.11  | 0.50 $\pm$ 0.18 |
| (Stem length)*                             | 0.74 $\pm$ 0.11  | 0.49 $\pm$ 0.18 |
| (Stem length) <sup>3</sup>                 | 0.73 $\pm$ 0.11  | 0.48 $\pm$ 0.19 |
| Surface area (Du Bois formula)             | 0.89 $\pm$ 0.05  | 0.71 $\pm$ 0.11 |
| Surface area (Meeh-Rubner-Heubner formula) | 0.87 $\pm$ 0.05  | 0.72 $\pm$ 0.10 |
| Surface area (Howland formula)             | 0.88 $\pm$ 0.05  | 0.73 $\pm$ 0.10 |

\* The second figure in each column represents the standard error of the coefficient  $r$ , obtained from the formula  $e(r) = \frac{1 - r^2}{\sqrt{n}}$

treatises on the theory of statistics, in general, however, there is a parallelism in the degree with which the former approaches  $\pm 1$  and the latter approaches 0 when correlation of the two variables is treated by the two methods.

The coefficients of correlation listed in table 8 show that the linear correlation is best between blood volume and body weight, blood volume and surface area, and blood volume and body length. On the other hand plasma volume shows poor agreement with any of these measurements. If the blood volumes are plotted as ordinates against the body weight as abscissae, points representing individual

TABLE 9  
*Ratio of blood volume to various body measurements*

| Patient                  | Blood volume | Blood volume<br>Weight | (Blood volume) $\times 10$<br>Area (Du Bois) | (Blood volume) $\times 10$<br>Area (Meeh) | (Blood volume) $\times 10$<br>Area (Howland) |
|--------------------------|--------------|------------------------|--|---|--|
| J G                      | 220          | 100.9                  | 1.264  | 1.099                                     | 1.233  |
| L S                      | 275          | 77.9                   | 1.159  | 0.997                                     | 1.179  |
| D M                      | 290          | 81.7                   | 1.280  | 1.047                                     | 1.186  |
| F B                      | 300          | 67.9                   | 1.169  | 0.936                                     | 1.047  |
| F T                      | 320          | 69.9                   | 1.143  | 0.975                                     | 1.087  |
| M A.                     | 320          | 66.5                   | 1.032  | 0.944                                     | 1.048  |
| J W                      | 340          | 78.0                   | 1.349  | 1.071                                     | 1.199  |
| E E                      | 345          | 93.2                   | 1.431  | 1.216                                     | 1.376  |
| L G                      | 355          | 55.6                   | 1.032  | 0.867                                     | 0.931  |
| C. K.                    | 370          | 112.1                  | 1.713  | 1.403                                     | 1.592  |
| L. H.                    | 385          | 74.0                   |  | 1.079                                     | 1.188  |
| S E                      | 410          | 61.0                   | 1.136  | 0.967                                     | 1.031  |
| D B                      | 440          | 64.1                   | 1.306  | 1.024                                     | 1.086  |
| R W                      | 445          | 78.3                   | 1.307  | 1.175                                     | 1.280  |
| W P                      | 455          | 66.0                   | 1.218  | 1.055                                     | 1.120  |
| J L                      | 500          | 74.1                   | 1.393  | 1.176                                     | 1.253  |
| A. B                     | 505          | 66.2                   | 1.295  | 1.095                                     | 1.144  |
| J P                      | 535          | 84.3                   |  | 1.511                                     | 1.409  |
| M. K.                    | 550          | 96.0                   | 1.725  | 1.444                                     | 1.571  |
| A. H.                    | 560          | 73.9                   | 1.412  | 1.220                                     | 1.275  |
| M C.                     | 565          | 68.4                   | 1.430  | 1.161                                     | 1.196  |
| M C.                     | 600          | 72.3                   | 1.515  | 1.230                                     | 1.266  |
| A H.                     | 620          | 85.0                   | 1.590  | 1.386                                     | 1.459  |
| J J                      | 655          | 79.6                   | 1.609  | 1.351                                     | 1.393  |
| Number of Observations   |              | 24                     | 22   | 24  | 24   |
| Arithmetical Mean        |              | 77.0 $\pm 2.6^*$       | 1.34 $\pm 0.04$                              | 1.14 $\pm 0.03$                           | 1.23 $\pm 0.03$                              |
| Standard Deviation       |              | 13.0 $\pm 1.9$         | 0.20 $\pm 0.03$                              | 0.16 $\pm 0.02$                           | 0.17 $\pm 0.02$                              |
| Coefficient of Variation |              | 17 $\pm 2$             | 15 $\pm 2$                                   | 14 $\pm 2$                                | 14 $\pm 2$                                   |

\* The second figure gives the standard error

determinations will lie on both sides of a straight line the equation for which is obtained from the coefficient and is expressed by the for-

mula  $BV = 61 Wt + 80$  A simpler formula, and one which for this series gives an approximation almost equally satisfactory, is obtained from the average of the ratios of blood volume to weight, as expressed in table 9, and is represented by  $BV = 77$  cc per kilogram

From a study of these values, it appears that body weight or surface area calculated by any one of the three formulas used gives a fairly close measure of blood volume even in the presence of such sharp deviations of these functions from the values expected on the basis of age as are offered in this group of patients For these ratios we have tabulated the values in individual observations (table 9) and have calculated the average figures, standard deviations, and coefficients of variation, with their standard errors Since the correlation of blood volume with surface area is not significantly closer than that of blood volume with body weight, there is no material advantage in stating the formula for obtaining the blood volume from surface measurement, since the latter is readily obtainable only by the use of other formulas such as those quoted, based on weight or weight-and-length measurements

While the multiplication of determinations may conceivably reduce these standard deviations and increase the accuracy of prediction of the figure for the total blood volume, it is equally likely that there will always remain a number of individuals who, by reason of a constitutional factor not sufficiently taken into account in formulas of this simplicity, will show values deviating more or less sharply from such limits Our reasons for believing this to be true are based on the fact that we obtained duplicate determinations of blood volume in the case of two patients, L G and M K, whose points on the plotted graph of blood volume per kilogram were located at a greater distance from the line of the regression equation  $BV = 61 Wt + 80$ —the one above, the other below it—than any other points in the scatter For L G we obtained the value 355 cc on admission, 355 cc 11 days later, for M K, 485 cc on admission, 550 cc 17 days later The first values in each case have not been included in the determinations subjected to analysis in this paper for the reason that at the time they were obtained both patients showed some clinical evidence of dehydration and it was thought that they might at the time be suffering a temporary disturbance in blood volume However, while they are justly

excluded from the set of "normal values" they still serve effectively to corroborate the second set of figures and eliminate the likelihood of gross technical error in their determination

Our results differ, then, from those previously reported by other authors mainly in three ways: our average values for blood volume per kilogram are considerably lower, the correlation between blood volume and various simple body measurements is closer than between plasma volume and those measurements, and finally, the scattering of our ratio values of blood volume per kilogram is considerably greater than that obtained particularly by Darrow, Soule, and Buckman (18). The first point of difference may be ascribed to our use of the carbon monoxide method as contrasted with their use of the dye method. When the measurement of the circulating blood volume depends on the quantitative administration of a foreign substance the loss of which from circulation, whether it be by diffusion out of the vascular system or by dilution in reservoirs of plasma in the small vessels as claimed by Whipple (14), would result in a raising of the analytical figure, it is difficult to see how our results can be too low. They may be too high, if muscle hemoglobin binds any considerable share of the respired carbon monoxide. The second point of difference rests also on the choice of method, since the carbon monoxide method determines the blood volume directly, it is independent of additional error from hematocrit readings. Finally, the wider scattering of our results may in all probability be ascribed to the variety of developmental and nutritional states represented in our series.

#### SUMMARY

1 A technique is described for the determination in infants of the circulating blood volume by the carbon monoxide method, based on the successful use of this method in tests on adults.

2 In comparison with the dye method, the carbon monoxide method gave results which were more uniform and showed a fair conformity with body weight.

3 In a small series of determinations of blood volume in patients less than two years of age, the correlation of blood volume to body weight, to surface area, and to body length suggested a normal inter-relationship of these measurements.

4 As an estimate of the circulating blood volume in infants, exclusive of the new-born group, the formula

$$\text{Blood Volume in cc} = (\text{Body Weight in kgm}) \times (77 \pm 13)$$

may be expected to give the correct figure in more than half the cases

#### BIBLIOGRAPHY

- (1) Muller, E, *Jahrb f Kinderh* 1910, lxxii, 176 Die Blut-und Hamoglobinnmenge und die Sauerstoffkapazität des Blutes bei gesund- und bei blassaussehenden Kindern
- (2) Zuntz, N, and Plesch, J, *Biochem Ztschr* 1908, xi, 47 Methode zur Bestimmung der zirkulierenden Blutmenge beim lebenden Tiere
- (3) Gréhant, N and Quinquaud, E, *J de l'anat et de la physiol* 1882-83, xviii, 564 Mesure de la Quantité de Sang contenu dans l'organisme d'un Mammifère Vivant *Id Compt rend Acad Sci*, 1882, xciv, 1450 Mesure du volume de sang contenu dans l'organisme d'un Mammifère Vivant
- (4) Haldane, J, and Smith, J L, *J Physiol*, 1899-1900, xxv, 331 The Mass and Oxygen Capacity of the Blood in Man
- (5) Douglas, C G, *J Physiol*, 1905, xxxiii, 493 A Method for the Determination of the Volume of Blood in Animals *J Physiol*, 1910, xl, 472 The Determination of the Total Oxygen Capacity and Blood Volume at Different Altitudes by the Carbon Monoxide Method
- (6) Salvesen, H A, *J Biol Chem* 1919, xl, 109 The Determination of Blood Volume by the Carbon Monoxide Method
- (7) Chang, H C, and Harrop, G A, Jr, *J Clin Invest*, 1928, v, 393 The Determination of the Circulating Blood Volume with Carbon Monoxide
- (8) Van Slyke, D D, and Robschey-Robbins, Frieda S, *J Biol Chem*, 1927, lxxii, 39 The Gasometric Determination of Small Amounts of Carbon Monoxide in Blood, and Its Application to Blood Volume Studies
- (9) Harrington, C R, and Van Slyke, D D, *J Biol Chem*, 1924, lxi, 575 On the Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement II
- (10) Van Slyke, D D, and Neill, J M, *J Biol Chem*, 1924, lxi, 523 The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement I
- (11) Stadie, W C, and Martin, K A, *J Clin Invest*, 1925, ii, 77 The Elimination of Carbon Monoxide from the Blood
- (12) Arnold, H R, Carrier, E B, Smith, H P, and Whipple, G H, *Am J Physiol*, 1921, lvi, 313 Blood Volume Studies V The Carbon Monoxide Method—Its Accuracy and Limitations
- (13) Keith, N M, Rowntree, L G, and Geraghty, J T *Arch Int Med*, 1915, xvi, 547 A Method for the Determination of Plasma and Blood Volume

- (14) Smith, H P , Arnold, H R , and Whipple, G H , Am J Physiol , 1921, lvi, 336 Blood Volume Studies VII Comparative Values of Welcker, Carbon Monoxide, and Dye Methods for Blood Volume Determinations Accurate Estimation of Absolute Blood Volume.
- (15) Lucas, W P , and Dearing, B F , Am J Dis. Child , 1920, xxi, 96 Blood Volume in Infants Estimated by the Vital Dye Method
- (16) Bakwin, H , and Rivkin, Helen, Am J Dis Child , 1924, xxvii 340 The Estimation of the Volume of Blood in Normal Infants and in Infants with Severe Malnutrition
- (17) Marriott, W M , and Perkins, J F , quoted in Marriott, W M , Am J Dis Child , 1920, xx, 461 Some Phases of the Pathology of Nutrition in Infancy
- (18) Darrow, D C , Soule, H C , and Buckman, T E , J Clin Invest , 1928, v, 243 Blood Volume in Normal Infants and Children
- (19) Area in sq cm =  $(\text{Wt. in kgm})^{0.423} \times (\text{Height in cm})^{0.725} \times 71.84$   
 Du Bois, D , and Du Bois, E F , Arch Int. Med , 1916, xvii, 863 Clinical Calorimetry X A Formula to Estimate the Approximate Surface Area If Height and Weight be Known  
 Area in sq cm =  $(\text{Weight in grams})^{\frac{1}{3}} \times 11.9$  The formula of Meeh and the coefficient of Rubner and Heubner are quoted by Benedict, F G , and Talbot, F B The gaseous Metabolism of Infants Carnegie Institute of Washington, 1914  
 Area in sq cm =  $0.483 (\text{Weight in grams}) + 730$  Quoted by Benedict and Talbot
- (20) Yule, G U , An Introduction to the Theory of Statistics London, Charles Griffin and Co , 1927, 8th Edition



# AN APPARATUS FOR THE PROLONGED ADMINISTRATION OF ARTIFICIAL RESPIRATION

## I A DESIGN FOR ADULTS AND CHILDREN

BY PHILIP DRINKER AND LOUIS A SHAW

*(From the Departments of Ventilation and Illumination and of Physiology, Harvard School of Public Health, Boston, Mass.)*

(Received for publication December 17, 1928)

### INTRODUCTION

Up to the present time, artificial respiration has generally been administered either (a) by manual methods or (b) by forcing air under pressure into and out of the lungs by means of an insufflating apparatus

The first method was brought to its present state of development by Sir E. A. Sharpey Schäfer. The obvious defect in this method lies in the fact that the chest is never inflated, it is only deflated and then allowed to return to the normal position through its inherent elasticity. This process is in the reverse direction to that which is normal and if, for any reason, the tone of the respiratory muscles becomes impaired, the chest will refuse to return to the normal position, thereby creating a situation in which adequate ventilation is rendered impossible.

The second means of artificial respiration has reference to positive pressure devices such as the bellows (Keith, 1909) and to the lung motor and pulmotor, which force air in and out of the lungs, usually through a face mask fitted with inlet and outlet valves. Among rescue squads in this country and Canada, this general method has been discredited and discarded (Engineering Committee of the Conference on Electric Shock, 1928).

A wholly new method has recently been devised by Thunberg (1927). The patient is placed in a chamber in which the pressures are alternately raised and lowered about 55 mm of mercury. Since the pressure changes are equal on both sides of the thoracic wall

there is no movement of the chest. The alternate rarefaction and compression of a fixed volume of air causes a volume flow which is proportional to the changes in pressure and which thus produces adequate gas exchange in the lungs.

A fourth method was described by Doe (1889). This apparatus was devised for resuscitating asphyxiated children. It consisted of a small box, over an opening in which a rubber dam was stretched. The infant was so placed in the box that his nose and mouth were held against a hole in the rubber dam. Through a tube connected to the box a person outside alternately blew in and sucked out air. Although there is no reason to doubt the efficacy of such a method, the task of supplying the pressures in this way for long periods must have been arduous and the results rather uncertain.

In South Africa, Steuart (1918) suggested the use of a mechanically operated device for inducing artificial respiration in children with anterior poliomyelitis. To quote the author: "The principle used is to place the child's thorax and abdomen in a rigid air-tight chamber communicating with a large bellows, which periodically causes a partial vacuum in the box." Either positive or negative pressures, or both, were obtainable and could be regulated as to frequency and amount. Apparently the author carried his work no further (so far as the literature indicates) than the suggestion of this device.

Through newspaper reports, our attention was brought to a device patented (1928) by Eisenmenger of Vienna. We had an opportunity recently to make experiments with this apparatus on normal men and also to serve as subjects. A rigid leather dome-shaped piece lined with rubber is placed over the patient's chest, extended about over the region from the clavicle to the umbilicus, and strapped tightly in position. Alternate positive and negative pressures inside the dome are applied by an electrically driven diaphragm pump. Although the dome-shaped cover is not air-tight at any time, the pump produces any desired pressure up to about 400 cm of water, either positive or negative.

The apparatus which we have developed (figs. 1 and 2) is based on principles somewhat different from any of those mentioned. The patient's body is entirely enclosed in a cylindrical sheet-metal tank sealed at one end. At the other end is a flat lid, to which is attached

a rubber collar. The patient's head and part of his neck protrude through the collar, the head lying upon an adjustable support outside the tank. Since the patient is thus enclosed in an air tight chamber, the pressure around the body may be alternately raised and lowered while the head always remains at atmospheric pressure. When the

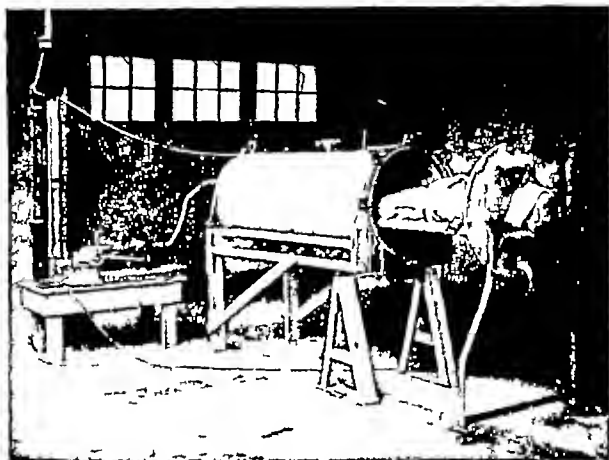


FIG. 1 MECHANICAL RESPIRATOR, SHOWING TANK WITH PUMP MECHANISM CONNECTED BY FLEXIBLE TUBE AT FOOT END

The collar and head rest are in place and the patient is ready to be pushed into the tank.

pressure in the tank is lowered, the chest expands and air rushes into the lungs. When the pressure is raised, the chest is compressed and air is forced out of the lungs. By this method, movement of the chest is induced in such a manner as to simulate the natural respiratory movements.

#### DESCRIPTION OF NEW MECHANICAL RESPIRATOR

The body of the respirator is made of metal (preferably sheet iron) welded at all joints. Rails in the form of "channels" are welded to the

inside The patient lies on a mattress supported on an angle iron frame to which springs are hooked on all four sides This bed frame is fastened rigidly to the lid of the tank, the bed and lid rolling in and out of the tank on wheels The rubber collar through which the patient's head protrudes is fastened to the outside of the lid The

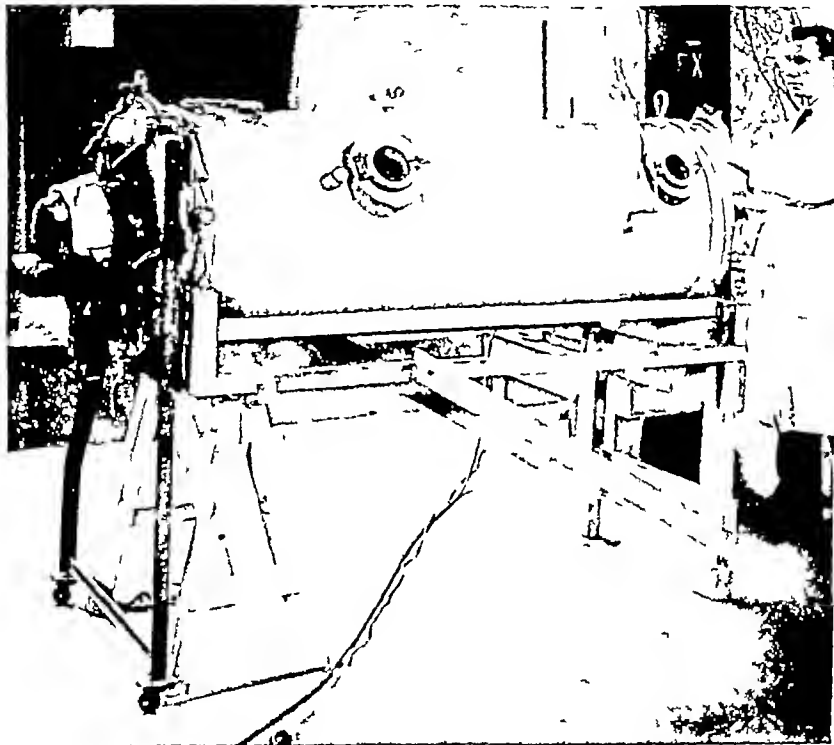


FIG 2 A PATIENT IN THE RESPIRATOR

Note the position of the port-holes Attendant has his hands on the dial of the thermostat which controls the temperature and humidity of the air in the tank

patient can thus be taken out occasionally for examination without removal of this collar (fig 3) To transfer a patient from a bed or stretcher to the tank requires about two minutes If necessary, manual respiration can be carried on during this interval

The lid of the tank is clamped tightly against the body of the tank by means of refrigerator locks On the inner surface of the lid there is a small  $\Lambda$ -shaped rim, which fits into a seamless rubber gasket dove-

tailed into the body of the tank. This arrangement assures a sufficiently tight seal on clamping the lid in place

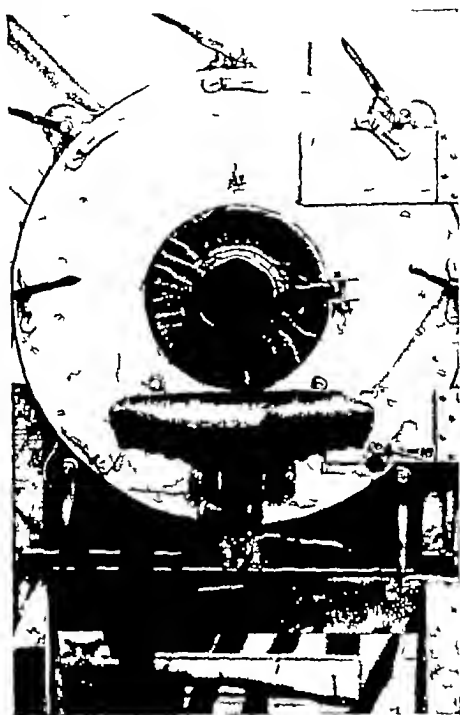


FIG 3 HEAD END OF TANK SHOWING ADJUSTABLE HEAD REST AND RUBBER COLLAR

In future, the collars will be molded in a single piece and bolted to the lid of the tank by metal rings with wing nuts

The tank can be rotated about 75 degrees in both directions and the foot end can be raised or lowered about 15 degrees with the horizontal. The chief purpose of these maneuvers is to give the patient a change in

position If necessary, sides can be placed on the bed to keep the patient in position when the tank is rotated

The body of the tank is equipped with marine-type glass portholes for observing the patient and with numerous small holes (pipe taps) for inserting thermometers, attaching manometers, connecting pneumographs, blood pressure cuff, or stethoscope For taking x-ray photographs of chest movements, the glass of the porthole is replaced by a sheet of thin aluminium permeable to x-rays

The pumps (fig 1) which produce the pressure consist of two small universal blowers<sup>1</sup> The valve mechanism controlling the pressure alternations is made up of two concentric cylinders, the outer one is stationary and the inner one driven by a worm gear connected through a variable speed motor Holes are cut at certain intervals in both cylinders and these holes in turn lead out on one side to the pumps and on the other to the tank The valves, driving gear, and cocks are so arranged that the pumps may be used in series, in parallel, or singly Either pump may be disconnected and replaced by a new one without stopping the apparatus Any combination of pressures, positive or negative, up to about 60 cm of water can be applied and any number of pressure changes (i e , breaths) from 10 to 40 per minute are obtainable

The manometer which indicates pressures within the tank is filled with water colored red for visibility, the scale measures in half centimeters above and below the zero point (atmospheric pressure) In controlling pressures, it is difficult to read both limbs of the manometer at the same time We therefore use the rising limb only and, by measuring in half centimeters (thus doubling the actual reading) get the total excursion

The rubber collar (fig 3) was developed by the Hood Rubber Company The diameter of the hole in the collar for the neck and of the hole in the lid of the tank through which the subject's head must pass and to which the collar is fixed were taken from figures supplied us by

<sup>1</sup> Manufactured by the Electric Blower Company, Boston, Mass These blowers were chosen because they are designed for continuous service and deliver a considerable volume of air against 75 to 100 cm of water pressure Although these pumps function satisfactorily, their noise is a drawback in hospital work We expect shortly to substitute pumps of more silent operation

the Cluett Peabody Company, collar manufacturers, and by the Knox Hat Company. Dimensions for the smaller collar sizes were taken from our own measurements on infants and children.

Excessive movement of the rubber collar from the pressure changes is prevented by semi lunar discs of thin metal placed on both sides of the collar. This permits the use of thin rubber about the neck. At present, we are using a series of six collar sizes, which seems adequately to cover variations in neck sizes from children two years of age to adults.

The rubber collars must withstand continuous pressure changes of from 0 to 60 cm. of water without appreciable leakage, they must be actually air-tight at low pressures, and, at the same time, they must not be uncomfortable. Up to the present, all of our collars have been built by hand, but we hope soon to have them moulded in one piece, which would materially reduce their cost and increase their uniformity.

#### PRESSURES REQUIRED TO INDUCE BREATHING

Our chief purpose in making this apparatus was to secure a device for treating patients suffering from protracted cases of respiratory failure, such as are induced by severe carbon monoxide poisoning, electric shock, morphine poisoning, poliomyelitis, and the like. The important question of how much pressure is needed to induce breathing in pathologic cases was estimated by using a number of normal men and women as subjects and observing their reactions when the pumps were working against various pressures.

Table 1 gives the results of a series of experiments on normal men and women, chosen at random from the laboratory personnel in the building. The plot of these data (fig. 4) shows that there is a very considerable variation in individual reactions and experiments like nos. 11 and 12 indicate that the same subject does not necessarily react in the same way in two experiments under approximately the same pressures. As the normal minute volume itself varies as much as 10 or 20 per cent, it seems probable that the scattering of the points about the dotted line of figure 4 are within the normal expected variation.

In order to determine approximate threshold pressures on a normal subject, one observes the manometer and gradually increases the

TABLE 1  
*Changes in minute volume resulting from various pressures*

| Experiment | Subject | Vital capacity | Normal minute volume | Average plus and minus pressures applied | Resulting minute volume | Increase above normal | Approximate threshold pressure         |
|------------|---------|----------------|----------------------|--|-------------------------|-----------------------|--|
|            |         | <i>liters</i>  | <i>liters</i>        | <i>cm water</i>                          | <i>liters</i>           | <i>per cent</i>       | <i>cm water</i>                        |
| 1          | A (m)   | 6 1            | 10                   | 7 6                                      | 8 4*                    | -15 7                 | 10                                     |
| 2          |         |                |                      | 10 2                                     | 10 0*                   | 0                     |  |
| 3          |         |                |                      | 12 0                                     | 12 6*                   | 25 7                  |  |
| 4          |         |                |                      | 19 0                                     | 16 3*                   | 63 0                  |  |
| 5          |         |                |                      | 21 0                                     | 23 5*                   | 135 0                 |  |
| 6          |         |                |                      | 30 7                                     | 40 0*                   | 300 0                 |  |
| 7          | B (m)   | 5 8            | 5 8                  | 4 3                                      | 5 5                     | -5 0                  | 10                                     |
| 8          |         |                |                      | 9 1                                      | 5 8                     | 0                     |  |
| 9          |         |                |                      | 11 6                                     | 7 7*                    | 33 0                  |  |
| 10         |         |                |                      | 14 0                                     | 10 8                    | 86 2                  |  |
| 11         |         |                |                      | 21 3                                     | 11 6*                   | 100 0                 |  |
| 12         |         |                |                      | 21 6                                     | 17 2                    | 193 0                 |  |
| 13         | C (m)   | 5 1            | 9                    | 6 3                                      | 10 6                    | 17 8                  | 6                                      |
| 14         |         |                |                      | 11 5                                     | 13 9                    | 54 0                  |  |
| 15         |         |                |                      | 19 1                                     | 19 0                    | 111 0                 |  |
| 16         | D (f)   | 3 2            | 5                    | 7 9                                      | 4 4                     | -12 0                 | 8                                      |
| 17         |         |                |                      | 9 7                                      | 9 8*                    | 96 0                  |  |
| 18         |         |                |                      | 11 6                                     | 10 0                    | 100 0                 |  |
| 19         |         |                |                      | 17 1                                     | 14 8*                   | 196 0                 |  |
| 20         |         |                |                      | 21 5                                     | 22 2                    | 340 0                 |  |
| 21         | E (m)   | 4 5            | 8 2                  | 8 2                                      | 15 4*                   | 87 5                  | 4                                      |
| 22         |         |                |                      | 16 4                                     | 23 6*                   | 188 0                 |  |
| 23         | F (m)   | 5 6            | 8                    | 5 0                                      | 11 6                    | 45 0                  | 2 noticeable<br>20 complete<br>control |
| 24         |         |                |                      | 9 5                                      | 16 2                    | 100 0                 |  |
| 25         |         |                |                      | 15 1                                     | 19 6                    | 145 0                 |  |
| 26         |         |                |                      | 20 3                                     | 34 7                    | 334 0                 |  |
| 27         | G (f)   | 2 4            | 6 6                  | 6 8                                      | 12 7*                   | 93 0                  | 4                                      |
| 28         |         |                |                      | 17 3                                     | 22 0*                   | 233 0                 |  |

\* Minute volumes determined in the course of taking Benedict metabolism records  
 Other values determined spirometrically (Drinker, 1927)

pressures—positive and negative—by turning the proper valves. When there is too little pressure to be effective, the subject's breathing is not in perfect synchronism with the alternations of the pump and the excursions of the water in the manometer are therefore very irregular

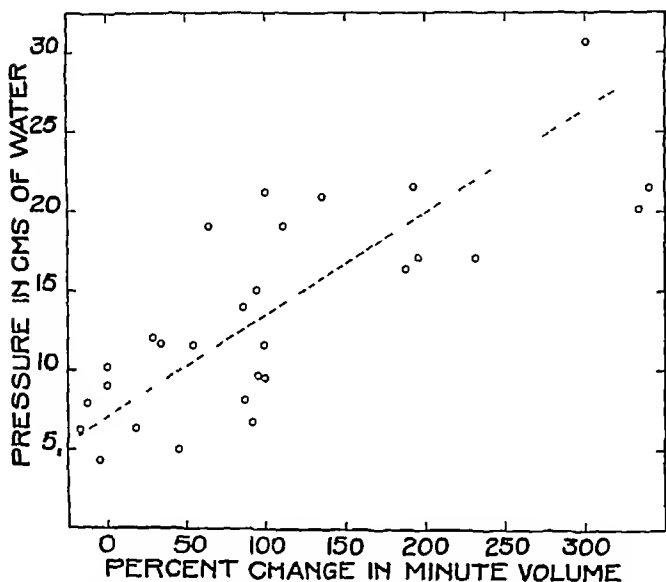


FIG. 4 THE DATA OF TABLE 1 PLOTTED AS A MEANS OF DETERMINING APPROXIMATE THRESHOLD PRESSURES—PRESSURES AT WHICH THE RESPIRATORY MOVEMENTS OF THE AVERAGE SUBJECT FALL INTO PERFECT SYNCHRONISM WITH THE PUMP

Where the dotted line passes through zero is the approximate threshold pressure

As the pressures are increased, a point is reached (generally at about 5 to 10 cm) when the subject falls into rhythm with the pump and remains so, no matter how much the pressure is increased subsequently. For one man of normal physique (subject F, table 1) about

18 to 20 cm were required before the pump completely controlled his breathing. Another subject, A, a tall thin man, fell in at about 10 cm. With refractory subjects like F, synchronism with the pump is generally achieved by starting with pressures of about 18 to 20 cm and then rapidly coming down to 5 to 10 cm, which, from figure 4, seems to give about the average threshold range.

The conditions encountered in the tank are quite unlike anything one experiences normally. The head is at atmospheric pressure and the chest, diaphragm, and abdomen are alternately under positive and negative pressures. Of the two, negative pressure (inducing inspiration) is much the more difficult to resist.

There is no certain way of predicting the optimum combination for any individual. The most satisfactory procedure seems to consist in having all the controls as flexible as possible and quickly exploring the likely pressure ranges until a reasonable one is found to fit the patient.

#### THE EFFECT OF ALTERNATE POSITIVE AND NEGATIVE PRESSURES UPON SPEECH

Another method of determining threshold pressures for normal subjects is given by the effect of pressure changes upon speech. A new subject—especially if he understands the apparatus—usually enters the tank with a certain degree of skepticism—he doubts the effectiveness of such small pressure changes. As the pressures are gradually increased, he is likely to attempt to talk or laugh and, as either function is cut off abruptly by inspiration, one can easily tell when the threshold pressure is approached. For the majority of subjects, speech is seriously hindered at pressures of about 5 to 10 cm and is almost wholly prevented at pressures of about 20 cm or more. This, of course, applies to the inspiration phase only.

#### PRESSURES EXERTED IN ARTIFICIAL RESPIRATION BY THE SCHAFER METHOD

At our suggestion, Mr. T. J. Shaughnessy, supervisor of resuscitation and instruction in the Consolidated Gas Company of New York, was so good as to have his rescue squads, numbering altogether about 45

men, perform the Schäfer prone pressure method on the platform of a spring scale which registered the pressure in pounds applied by the operator. All the subjects had repeatedly used the Schäfer method in actual resuscitation work in and about New York City. The results of the experiment, plotted in figure 5, show that the pressures applied ranged from about 41 to 63 kilos and varied roughly with the weight of the operator.

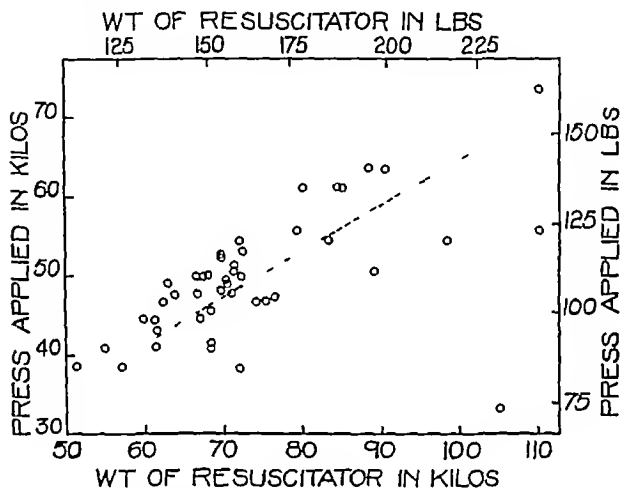


FIG 5 A COMPARISON OF THE WEIGHTS AND PRESSURES APPLIED BY 45 EXPERIENCED "PRONE PRESSURE RESUSCITATORS" FROM THE RESUSCITATION CRFWS OF THE CONSOLIDATED GAS COMPANY OF NEW YORK  
Courtesy of Mr T J Shaughnessy

This range is somewhat above the 23 to 41 kgm (50 to 90 pounds) given by Keith (1909) as practical working figures in applying the Schäfer method.

Mr Shaughnessy's data are of interest to us because they show what relatively great pressures must be applied over a small area (the palms of two hands) in practical resuscitation work. In this case, of course, positive pressure only (forcing expiration) is exerted.

## THE EFFECT OF THE RESPIRATOR ON METABOLISM

If a subject is passive and readily falls into rhythm with the pump, it is reasonable to expect a consequent reduction in metabolism, for the normal muscular work of breathing is supposedly being performed in large part by the pump. A considerable series of careful experiments upon normal men and women did not, however, bear out this expectation. The results showed that, for the most part, metabolism was not affected significantly unless threshold pressures were considerably exceeded, then increases of 10 to 20 per cent were obtained, owing, apparently, to the muscular work of resisting over-ventilation.

## OVER-VENTILATION AND ITS SIGNIFICANCE

It is impossible to produce on a normal subject more than a slight degree of apnea by the Schafer method. This method also has little effect upon a subject who has previously breathed forcibly for some minutes and has already become apneic. The mechanical respirator, on the other hand, offers a convenient method for producing apnea, for forcing a resistant or apneic subject to breathe, and for studying recovery from apnea.

Figure 6 shows a Benedict metabolism record of subject A in the tank. He first breathed normally, then with one pump running at 20 cm pressure, then with two pumps at about 30.7 cm. The record shows the subsequent apnea which extended over a period of some four minutes. There was, clearly enough, an appreciable consumption of oxygen during these four minutes, although the respiratory movements were negligible.

In making this particular record, the rubber flap valves ordinarily used in the Benedict apparatus to control inspiration and expiration were removed. Instead we used a small blower or "impeller" devised by Collins for use with the apparatus when the subject is breathing deeply, as in performing muscular work. In this way, oxygen at atmospheric pressure is brought continually to the subject, so that, as the oxygen in the lungs is converted into carbon dioxide, more oxygen is drawn in to replace it. This process can take place with no visible chest movement.

It may well be objected that the data obtained upon healthy sub-

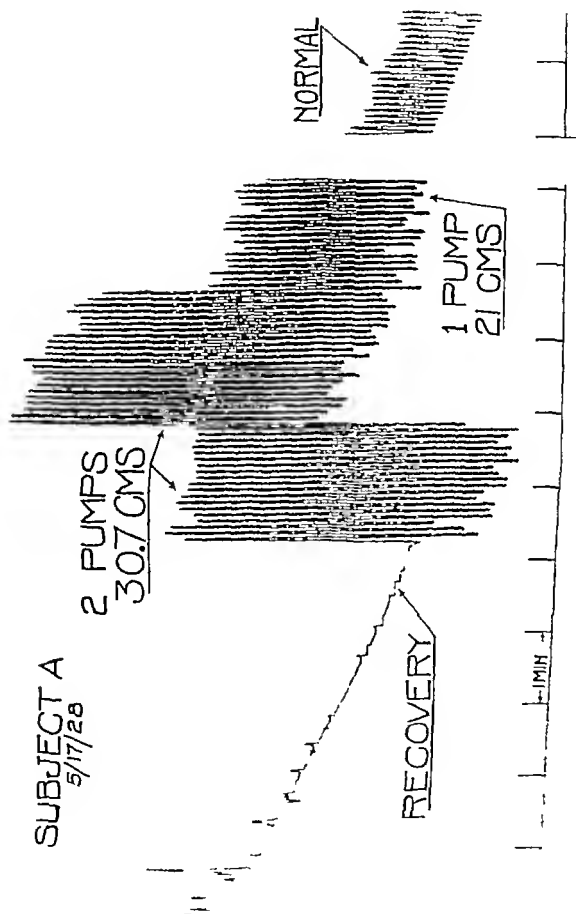


FIG. 6. IMPROPER ON A NORMAL MAN OF THE MECHANICAL RESPIRATOR, AS SHOWN BY THE BENEDICT PORTABLE METABOLISM APPARATUS

Record reads from right to left. Abscissa represent time and ordinates oxygen consumption. Note the pronounced oxygen consumption in the recovery (apnea) after the pumps had been shut off.

jects are not valid and may only reflect the fact that our subjects (table 1) find it easier to cooperate with the respirator than to assume a passive attitude. Such an objection, however, may safely be disposed of by considering the ease with which the subject can be over-ventilated to any desired degree (fig 6). It has been demonstrated by

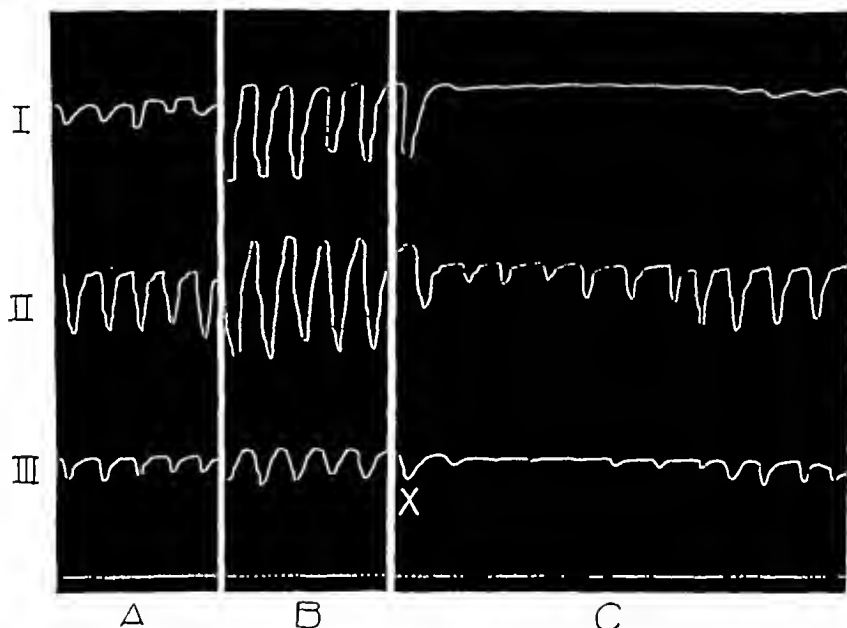


FIG 7 PNEUMOGRAPHIC RECORDS TAKEN ON NORMAL SUBJECT

I, Porter pneumograph is placed around chest at level of the fourth rib, II, pneumograph is 2.5 cm. below the tip of the xyphoid, and III, pneumograph is 13 cm. below position in II. *A* represents normal breathing, *B* forced breathing with the pump delivering an average of +12 and -10 cm. of water pressure, and *C* recovery after the pump had been shut off at point *X*. Time intervals = 1 second.

laboratory experiments upon mammals that over-ventilation is resisted by a muscular effort no less pronounced than that which follows under-ventilation.

In order to prevent over-ventilation and the consequent development of alkalemia in the case of a patient suffering from respiratory failure, it is best to collect the expired air from time to time in a spirometer and determine the minute volume. The latter should then be

regulated to conform with the average respiratory volume per minute per kilogram of body weight for the particular individual

#### CHEST AND DIAPHRAGMATIC MOVEMENTS

##### *Pneumograph records*

In order to study the relative movement of the chest and diaphragm in natural and in mechanically forced breathing, three Porter pneumographs were placed on subject A, connected so as to write separate records on a kymograph equipped with a second marker

The first pneumograph, I, was placed at the level of the fourth rib, the second, II, 2.5 cm below the tip of the xyphoid, and the third, III, 13 cm below the second. A record of normal breathing was then made (fig 7, A) with the subject lying at rest in the tank. Next the pump was run at pressures of +12 and -10 cm giving the record shown in figure 7, B. Finally, the pump was stopped and the period of recovery from the slight apnea produced is shown in figure 7, C.

Compared with normal breathing, breathing induced by the mechanical respirator showed a marked increase in chest movement, particularly in movement of the upper part of the chest. Directly after the pump was stopped, there appeared to be practically no movement in the first and third pneumographs and a slight movement in the second. As recovery progressed, diaphragmatic breathing (the third pneumograph) became more pronounced and, after about one minute, was practically normal. The upper pneumograph shows that normal breathing had not been resumed about one minute after the pump was stopped.

##### *X ray record*

A portable x ray machine was arranged so that the tube was directly over the central porthole, near the head end of the tank (fig 2). By replacing the glass of the port with thin aluminium, x-ray photographs of the subject's chest movements could be taken. The subject in this case was an athletic man 27 years of age, apparently in excellent health. His vital capacity was 4.5 liters.

Dr Merrill C. Sosman<sup>2</sup> was kind enough to take the pictures for us and commented upon the resulting plates as follows:

<sup>2</sup> Roentgenologist in-chief, Peter Bent Brigham Hospital, Boston.

Films of the chest on deep voluntary inspiration and expiration show a maximum movement of the diaphragms 8.8 cm, with a change in diameter of the chest of 3 cm

Films of the chest in the respirator, one at 46 cm of water, negative pressure (inspiration), and the other at 39 cm of water, positive pressure (expiration) show a change of 10.5 cm in the height of the diaphragms and a much more marked change in the width of the chest. The latter cannot be measured, as at expiration the edges of the chest are beyond the limits of our largest film. It is estimated to be about 6 cm. A comparison of the two sets of films shows that forced inspiration and expiration cause more change in aeration of the lungs than do voluntary inspiration and expiration.

#### THE APPARATUS USED AS A MEANS OF RECORDING RESPIRATORY MOVEMENTS

When the subject lies at rest in the respirator breathing naturally without the mechanical assistance of the pump, the expansion of the chest caused by inspiration raises the pressure of the air in the respirator and, conversely, the deflation of the chest caused by expiration lowers the pressure. If now a manometer of high sensitivity be connected to the respirator, the respiratory movements can be easily followed. The sensitivity of such a manometer may be greatly augmented by tilting it at an angle of 5 or 6 degrees with the horizontal and by using a liquid of low specific gravity, such as ether.

By treating our respirator as a body plethysmograph, the respiratory movements may be recorded volumetrically. This may be effected by connecting the respirator with a Krogh spirometer of 5 or 6 liters capacity, with a writing point in apposition with a kymograph. At inspiration, a volume of air equivalent to that inspired is displaced into the spirometer by the expansion of the chest and at expiration the air is again withdrawn from the spirometer by the contraction of the chest. The alternate movements of the spirometer lid writes a record both of respiratory movements and of respiratory volumes. The accuracy of the latter is directly proportional to the sensitivity of the spirometer and inversely proportional to the volume of air surrounding the body, since a certain slight pressure must be built up in the plethysmograph to overcome the inertia of the spirometer.

This method of recording respiration has been previously used by Shaw (1928) and also by Binger and Davis (1928).

Since the tank can be changed at will from a mechanical respirator to a plethysmograph, it gives a convenient and clinically practical method of observing in pathologic cases whether the subject's normal breathing is being resumed

#### CONTROL OF TEMPERATURE AND HUMIDITY

A thermostat for controlling the temperature and humidity of the air in the tank is screwed in at the foot end near the top. When the tank is completely shut—when the pumps are not running and no air is being introduced from outside—the patient's body heat raises the temperature within to an uncomfortable degree, if some sort of thermostatic mechanism is not provided. In our apparatus, the air within the tank is circulated through a can of ice, which serves both as a dehumidifier and a cooling agent. The small blower, producing the air circulation, is controlled by this thermostat.

When the pumps are running, the air is continually being changed—the air withdrawn from the tank is exhausted and blown into the room, without finding its way directly into the tank again. Then thermostatic control is not needed.

#### DISCUSSION

We recently had an opportunity to apply our respirator (1929) in a case of intercostal paralysis from poliomyelitis. The patient, a child of eight years, was in the machine almost continuously for a period of 122 hours, at the end of which time death occurred, resulting apparently from cardiac failure brought on by an extensive bronchopneumonia in the right lung. Examination of the lungs at autopsy showed no evidence of trauma from overinflation, nor was any other form of damage to the patient from this prolonged application of artificial respiration observed. During the time the child was in the respirator, she was able to talk, sleep, and take nourishment while the pumps were running.

This case and another described by Petrén and Sjövall (1926) indicate that respiratory failure in cases of poliomyelitis can be prevented and encourage the hope that recovery may follow in certain cases which would otherwise terminate fatally.

Our respirator in its present form, like Thunberg's Barospirator, is a rather cumbersome and complicated apparatus—it is a clinical tool to be used in hospitals under the direction of competent clinicians. For emergency rescue work, it cannot displace the Schafer method of manual resuscitation.

We are indebted to the gas and electric companies affiliated with the Consolidated Gas Company of New York through their Committee on Resuscitation and Related Activities for the funds which enabled us to bring this apparatus through the experimental stages.

We wish to express our thanks to Mr F C Christensen, Superintendent of the Machine Shop of the Harvard Medical School, who designed and manufactured the valve mechanism of this apparatus. We are especially indebted to him for the many improvements which he has introduced since the first experimental respirator was built.

#### SUMMARY

The literature on mechanically operated devices for the application of artificial respiration is briefly reviewed and a new type of respirator is described and illustrated. This respirator consists in a metal tank, sealed at one end, in which the patient is placed, his head protruding through a rubber collar attached to the open end of the tank. By electrically operated pumps, the atmosphere within the tank is placed alternately under positive and negative pressure, positive pressure inducing expiration and negative pressure inspiration. The pressure changes per minute can be varied from 10 to 40 and any combination of pressures, positive or negative, from approximately 60 cm of water to atmospheric pressure can be obtained. The average threshold pressures—those which induce breathing against the subject's will—are found to be about 5 to 10 cm of water for normal men and woman. Apnea can be produced by means of the respirator and an apneic subject can be made to breathe deeply.

By turning a valve at the foot end, the respiration tank is converted instantly into a plethysmograph. If a suitable spirometer is attached, graphic records of breathing can be made and the patient's ability to breathe without assistance determined.

## BIBLIOGRAPHY

- Binger, C A L , and Davis, J S , Jr , *Proc Soc Exp Biol and Med* , 1928, xxv, 607 A Body Plethysmograph for the Study of Respiratory Movements in Human Beings
- Doe, O W , *Boston Med and Surg J* 1889, cxx, 9 Apparatus for Resuscitating Asphyxiated Children
- Drinker P , and McKhann, C F , *J Am Med. Assoc* , 1929, xcii, 1658 The Use of a New Apparatus for the Prolonged Administration of Artificial Respiration I A Fatal Case of Poliomyelitis Drinker P , Thomson, R. M , and Finn, J L , *J Indust. Hyg* , 1927, ix, 331 Metal Fume Fever IV Threshold Doses of Zinc Oxide, Preventive Measures, and the Chronic Effects of Repeated Exposures
- Eisenmenger R , 1928 U S Patent No 1,670,301
- Engineering Committee of the Conference on Electric Shock, *J Indust Hyg* , 1928, x, 117 Recent Experience of the Public Utilities of the United States and Canada in the Use of the Schäfer Prone Pressure Method of Resuscitation in Cases of Electric Shock.
- Keith, A , *Lancet* 1909, I, 745, 825, 895 The Mechanism Underlying the Various Methods of Artificial Respiration
- Petrén K., and Sjövall E , *Acta med Scandinav* , 1926, lxi, 260 Eine Studie über die tödliche akute Form der Poliomyelitis
- Shaw, L A , *Am J Physiol* , 1928, lxxv, 158 Cutaneous Respiration of the Cat
- Steuart, W , *Med J So Africa*, 1918, xii, 147 Demonstration of Apparatus for Inducing Artificial Respiration for Long Periods
- Thunberg, T , *Abderhalden s Handbuch der biologischen Arbeitsmethoden*, 1927, Abt. 5, Teil 1, 561 Der Barospirometer Ein Apparat für künstliche Atmung nach einem neuen Prinzip



# THE INVOLUTION OF CUTANEOUS XANTHOMATA CAUSED BY DIETS LOW IN CALORIES

By A. C. CURTIS, U. J. WILE AND H. C. ECKSTEIN

(From the Departments of Internal Medicine, Dermatology and Physiological Chemistry  
University of Michigan Medical School, Ann Arbor)

(Received for publication November 2, 1928)

Since the demonstration of cholesterol crystals in xanthomata by Bazin (1) in 1869 and the subsequent finding of a hypercholesterolemia by Chauffard and Laroche (2), cutaneous xanthomata have been considered cholesterol tumors. It has been pointed out by Wile, Eckstein, and Curtis (3) that xanthomatous tumors do not have as much cholesterol in them as does the surrounding skin and that their presence does not necessarily depend upon a lipemia or a hypercholesterolemia. In this same study it was demonstrated that not more than 11 per cent of the tumors was neutral fat and 16 per cent of this was cholesterol. Major (4) has shown that xanthomatous nodules consist of neutral fat, fatty acids, lipoids, and cholesterol. It is a common observation that xanthomata associated with hyperglycemia are often seen in obese individuals and as these tumors consist of neutral fat, fatty acids, lipoids, and cholesterol they may represent deposits of fat in the skin serving the same purpose as other fat depots in the body. If such a premise be true these cutaneous deposits of fat should respond to dietary measures as other fat depots do. Such was shown in our first paper (3) to be true, at least in part. In order to investigate this hypothesis further we have studied three patients with xanthomata. The first two patients were sisters who developed xanthomatous lesions within six months of each other without an associated diabetes. The third patient had a severe form of cutaneous xanthomata and diabetes mellitus. All patients had a lipemia.

## CASE HISTORIES

*Case 1.* K. L., female, age 8, came to the hospital complaining of an eruption which began 1½ years ago as a glass pin head size yellowish hypertrophic growth on

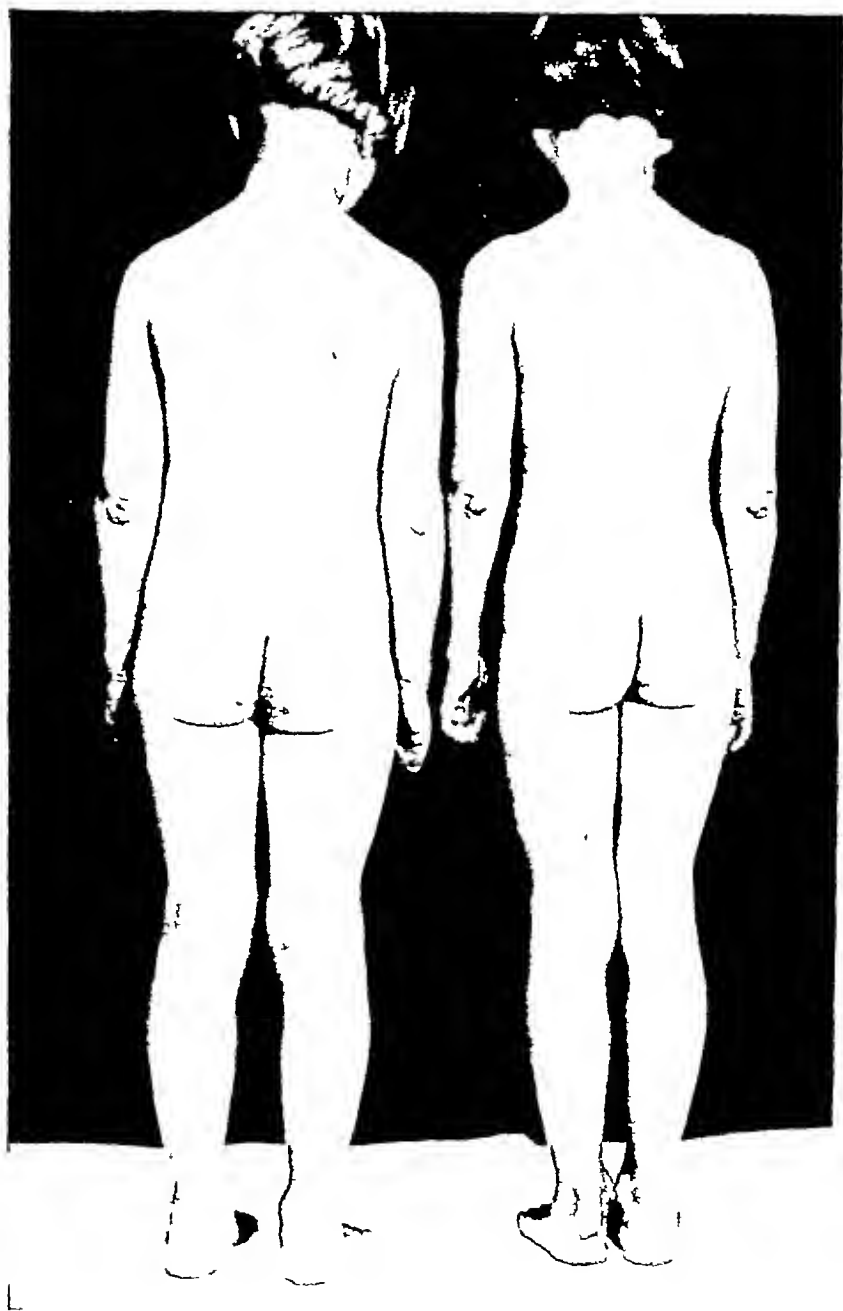


FIG 1 CASE 1, K L , AND CASE 2, M L , SHOWING THE DISTRIBUTION OF THE XANTHOMATOUS LESIONS AND THEIR NODULAR CHARACTER

the dorsal surface of both elbows. The nodules were yellow in color and painless. They slowly increased in size and in number until they involved all extremities and the trunk. Except for measles, mumps, whooping cough, and chickenpox, her past history is essentially negative. Neither has she had any symptoms referable to diabetes mellitus nor has there been any family history of the disease. On the anterior aspect of the body she presented three small lesions on the outer aspect of the right knee and two small lesions on the upper outer aspect of the left knee. These nodules varied approximately from 1 to 10 mm in diameter. They were sharply elevated from the surrounding skin, orange yellow in color, and firm to palpation. On the posterior aspect of the body the lesions were located over the elbows, in the gluteal cleft and over both tendo Achilles. The lesions for the most part were made up of groups of nodules varying in diameter from 1 to 15 mm. They were orange yellow in color, sharply elevated from the surrounding skin, and firm to palpation. (See fig 1.) Clinical diagnosis: xanthoma tuberosum. Pathological diagnosis: xanthoma tuberosum.

*Case 2* M L, age 7, a sister of K L, came to the clinic complaining of a small nodular eruption which had begun two years ago on the dorsal aspect of both elbows. The lesions slowly increased in size and in distribution until they were present over all extremities and the trunk. Her past history is negative except for measles, mumps, chickenpox and whooping cough. No symptoms of diabetes mellitus were present.

On the anterior aspect of the body she presented two small lesions on either side of the glabella which were approximately 1 to 10 mm in diameter. On the volar surface of the right wrist were two lesions, one approximately 15 mm and one approximately 2 mm in diameter. Over the outer aspect of both knees there were similar lesions slightly larger than those of the arms. Most of the lesions were orange yellow in color, sharply raised from the surrounding skin and firm to palpation. Over the posterior surface there were lesions on the neck which were verrucous and linear appearing something like a naevus. Over the elbows and in the popliteal space the lesions were made up of a confluence of smaller lesions varying in size from approximately 10 to 50 mm in diameter. Over the lower aspects of the tendo Achilles the lesions were somewhat smaller. (See fig 1.) Clinical diagnosis: xanthoma tuberosum. Pathological diagnosis: xanthoma tuberosum.

*Case 3* B B, male, age 42, came to the hospital because of an extensive eruption all over his body. Five years previously he noticed a small papular and nodular eruption on his shoulders and the extensor surfaces of his arms. The lesions were yellow, moderately firm and discrete. They rapidly spread until they involved the whole body with predilection for the buttocks, extensor surfaces of the arms and legs. Since then the lesions have persisted and are painful when traumatized.

The patient was an obese male (190 pounds) who presented over the trunk, elbows, knees, buttocks, and backs of the hands large numbers of typical tuberous xanthomatous lesions. The older lesions were quite hard and tender on pressure. The color of the lesions was a chamois yellow. The urine showed a 4+ sugar and the blood a definite hyperglycemia and lipemia.

Diagnosis: diabetes mellitus, obesity xanthoma tuberosum

#### METHODS

Case 1, K. L., and case 2, M. L., were hospitalized July 2, 1928, and placed on a diet consisting of 70 grams of protein, 150 grams of fat, and 100 grams of carbohydrate. Two basal metabolism determinations were done on each patient on successive days. The 24-hour basal calories were then calculated for both case 1, K. L., and case 2, M. L., from Sanford's Tables for their height, weight, age, sex, and basal metabolism. These determinations were followed by a glucose tolerance test which was repeated at the conclusion of the low-calorie diet periods. Daily weights were taken on each patient before breakfast clothed only in the standard hospital night-gown. Specimens for blood lipid determinations were drawn at the same time the first glucose tolerance test was done, 10 days later, and 30 days later. Routine daily urines were done. Case 3, B. B., was placed on four different types of diets. The first was the usual series of diets used by this hospital to determine the tolerance for glucose in diabetic patients, the second a definite reduction diet, the third a high fat and high-calorie diet, and the fourth a maintenance diet. Glucose tolerance tests were done at the beginning of the experiment and at its end. Daily urines were examined during all periods except the second. Only one urine analysis was obtained at the end of this period. Changes in his weight were recorded at the end of each dietary period.

Four blood lipid determinations were made, two on separate uncontrolled diets, one while on the high-calorie diet, and one while on the maintenance diet.

#### DATA

It will be seen from table 1, that case 1, K. L., and case 2, M. L., were placed on diets of 2030 calories. The 24-hour basal calories of case 1, K. L., were determined on successive days to be 1050 and 1090 calories. The 24-hour basal calories of case 2, M. L., were determined

on successive days to be 980 and 960 calories. It is evident then that this diet was well above their maintenance calories. They remained on this diet for 6 days. A slight increase in the firmness of the tumors and the depth of their color was noted. No marked change in weight occurred. For 8 days following this they were placed on diets of 50 grams of protein, 80 grams of fat, and 50 grams of carbohy-

TABLE 1

*The effect of various diets on xanthomatous lesions Case 1 A. L., and Case 2 M. L.*

| Date                 | Diet       |     |               |                  | Weight       |              | Blood fat      |                | Effect on tumors   |
|----------------------|------------|-----|---------------|------------------|--------------|--------------|----------------|----------------|--|
|                      | Protein    | Fat | Carbohydrates | Calories         | Case 1 A. L. | Case 2 M. L. | Case 1 A. L.   | Case 2 M. L.   |  |
|                      | gm         | gm  | gm            |                  | lbs          | lbs          | mgm per 100 cc | mgm per 100 cc |  |
| July 3-9             | 70         | 150 | 100*          | 2 030            | 53½<br>53½   | 52<br>52     | 1 383          | 1 330          | Increased in hardness.<br>Color deepened to orange yellow  |
| July 10-17           | 50         | 80  | 50*           | 1,172            | 53½<br>53½   | 52<br>52½    | 1,340          | 1,350          | No change  |
| July 18-26           | 40         | 20  | 40*           | 500              | 53½<br>52½   | 51½<br>51½   |                |                | No change  |
| July 27-<br>August 6 | 35         | 13  | 39            | 413              | 52½<br>48½   | 51½<br>48½   | 1 183          | 1,030          | Beginning involution                                       |
| August 7-10          | House diet |     |               | 22,500-<br>3 000 | 48½<br>55    | 48<br>52½    |                |                | Increase in size firmness and depth of color of all tumors |

\* Plus an unknown number of calories obtained surreptitiously from other patients.

drate with a total of 1120 calories. No effect was observed on the tumors and no change occurred in the patients' weight. We felt that this diet was too near their maintenance calories, even though they were up and about the ward, so they were placed on diets of 40 grams of protein, 20 grams of fat, and 40 grams of carbohydrate having a total of 500 calories. Two hours of exercise in the gymnasium was

done daily. One day after the diet was begun it was found that both patients had been given peanuts by their family and that during their wanderings about the ward, patients had given them candy, fruit, and food from the hospital trays. The patients hence were strictly con-

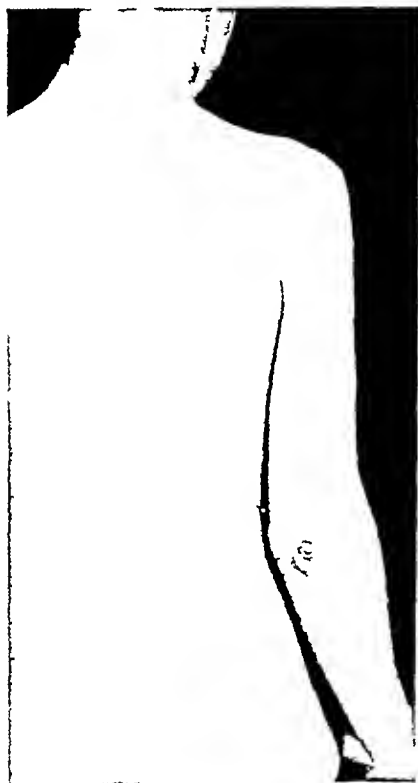


FIG 2, A



FIG 2, B

FIG 2 (A) CASE 1, K. L., SHOWING THE SIZE AND NODULAR CHARACTER OF THE XANTHOMATOUS LESION ON THE RIGHT ELBOW BEFORE TREATMENT (B) CASE 1, K. L., SHOWING THE DECREASE IN SIZE AND FLATTENING OF THE SURFACE OF THE LESION AFTER A SHORT PERIOD OF TREATMENT

fined to their rooms and for the first time a definite loss of weight occurred. Nine days later their diets were further reduced to 35 grams of protein, 13 grams of fat, and 39 grams of carbohydrate, having a total of 413 calories. A rapid loss of weight occurred. With the beginning of the weight loss there was also noted a definite softening of the

tumors The orange yellow color began to fade and a pink hue developed (See fig 2 and 3) It will also be seen from table 1 that the blood lipoids which had formerly been high, now showed a definite decrease

Unfortunately at this point we were notified that the children could only remain in the hospital 5 days longer so they were immediately put on house diets Their weight increased rapidly to a level greater



FIG 3, A



FIG 3, B

FIG 3 CASE 1, K L, (A) BEFORE TREATMENT AND (B) AFTER TREATMENT

Note the decrease in the size and fullness of the nodular eruption on the margins of the right and left popliteal spaces and the left heel

than at their entrance The lesions again became firm and the orange yellow hue returned

The urine examinations throughout the experiment were negative except for numerous fat droplets which disappeared while on the last low calorie diet The glucose tolerance curves (see fig 4) from these patients suggest an inability to tolerate large amounts of glucose

It will be seen from table 2 that case 3, B B, a mild diabetic, when placed on a series of desugarization diets in which the fat was constant and the calories were increased by the addition of glucose, experienced

an involution of his tumors and a loss of weight. This dietary procedure was followed by a diet consisting of 50 grams of protein, 50 grams of fat, and 200 grams of carbohydrate. While on this diet he lost 17 pounds in 30 days and his tumors continued to involute. He was then placed on a diet high in calories and fat for 30 days. This diet consisted of 55 grams of protein, 210 grams of fat, and 300 grams

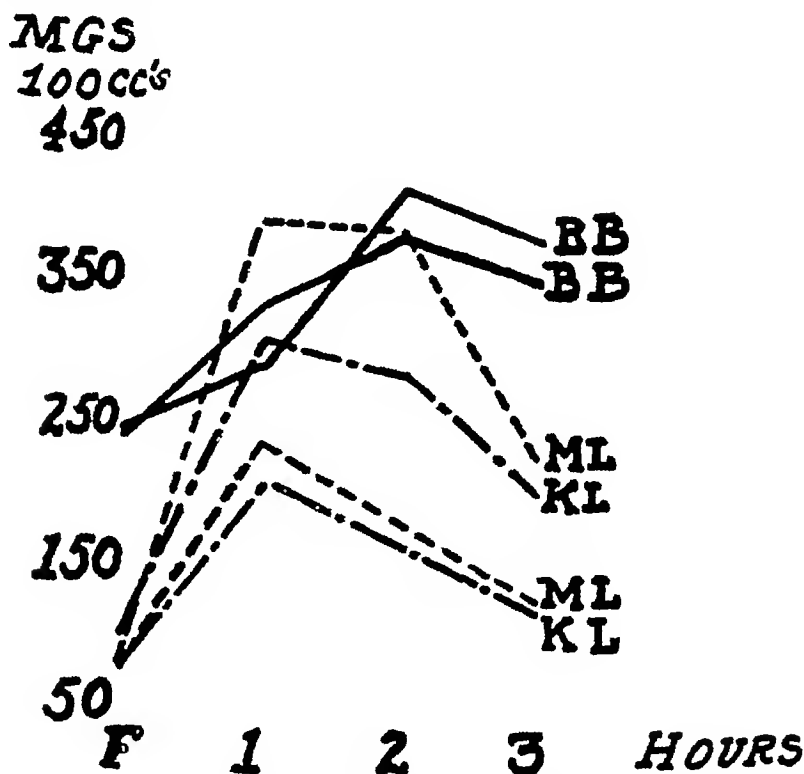


FIG. 4 THE TYPICAL DIABETIC INTOLERANCE FOR GLUCOSE OF CASE 3, B B AND THE ATYPICAL CURVES OF CASES 1, K L, AND 2, M L, ARE SHOWN IN THIS FIGURE

of carbohydrate with a total of 3310 calories. During this period he gained 8 pounds weight and had a reappearance of all his tumors. He also had a glycosuria and acetonuria. Following this he was given a maintenance diet consisting of 55 grams of protein, 210 grams of fat and 100 grams of carbohydrate with a total of 2510 calories. It will be noted that the fat of this diet is the same as the former diet but the

TABLE 2

*Effect of diets high in calories and low in calories on xanthomatous lesions Case 3, B B*

| Diets           |       |                |           | Duration of diet | Weight gain or loss     | Blood fat      | Effect on tumors  |
|-----------------|-------|----------------|-----------|------------------|-------------------------|----------------|---|
| Protein         | Fat   | Carbo-hydrates | Calo-ries |                  |                         |                |   |
| grams           | grams | grams          |           | days             |                         | mgm per 100 cc |   |
| House diet      |       |                | ?         |                  | Gain                    | 2 275          |   |
| 55              | 210   | 35             | 2 240     | 3                |                         | 2 215          | Beginning involution                                    |
| 55              | 210   | 50             | 2,300     | 3                |                         |                | Beginning involution                                    |
| 55              | 210   | 100            | 2 500     | 3                | Loss of 12 pounds       |                | Beginning involution                                    |
| 55              | 210   | 150            | 2,700     | 3                |                         |                | Beginning involution                                    |
| 55              | 210   | 175            | 2,800     | 3                |                         |                | Beginning involution                                    |
| 50              | 50    | 200            | 1 450     | 30               | Loss of 17 pounds       |                | Marked involution                                       |
| 55              | 210   | 300            | 3 310     | 30               | Gain of 8 pounds        | 2,416          | Reappearance  |
| 55              | 210   | 100            | 2,510     | 45               | Little change in weight | 1,175          | Complete involution                                     |
| 55              | 210   | 100            | 2 510     | 180              | Little change in weight |                | Complete involution, all tumors replaced by scar tissue |
| Unmeasured diet |       |                | ?         | 30               | Gained 5 pounds         | 2,225          | Reappearance of three tumors                            |

TABLE 3

*Influence of fat on blood lipids*

|                                    | Blood fat      | Increase |
|------------------------------------|----------------|----------|
| Patient B B case 3                 |                |          |
|                                    | mgm per 100 cc | per cent |
| Fasting                            | 2 300          |          |
| 2 hours after 100 cc. of olive oil | 2 630          | 14       |
| 4 hours after 100 cc. of olive oil | 2 769          | 16       |
| 6 hours after 100 cc. of olive oil | 2 337          | 2        |
| Patient S, normal blood fat        |                |          |
|                                    |                |          |
| Fasting                            | 439            |          |
| 2 hours after 100 cc. of olive oil | 467            | 6        |
| 4 hours after 100 cc. of olive oil | 600            | 37       |
| 6 hours after 100 cc. of olive oil | 508            | 13       |



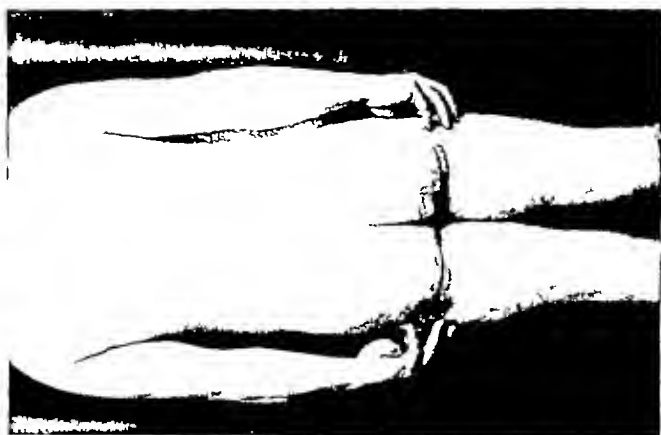


FIG 5, C

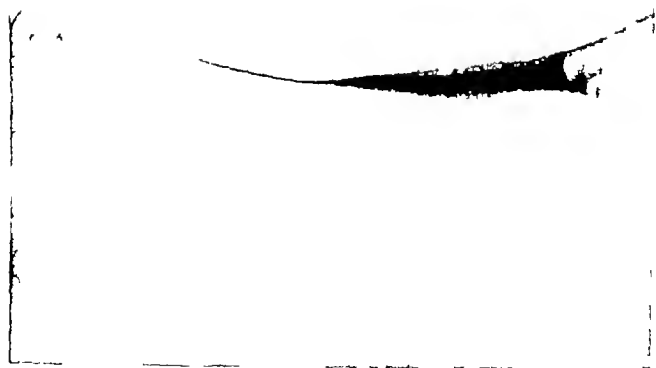


FIG 5, B



# THE SURFACE TENSION OF BLOOD SERUM, AND THE DETERMINATION OF THE SURFACE TENSION OF BIOLOGICAL FLUIDS

BY HENRY N HARKINS AND WILLIAM D HARKINS

*(From the Department of Chemistry, University of Chicago Chicago)*

(Received for publication January 4, 1929)

## INTRODUCTION

This investigation was undertaken in order to obtain a more exact knowledge of the surface tension of undiluted blood serum, and its variation under various clinical conditions, than is given by the work reported earlier by others (1-12) It was found that practically all previous investigators have made errors in the experimental procedure or in the calculation of their results, usually in both, of such a high magnitude as to make their data almost entirely worthless Thus a new field is opened up for future investigation

The mean value obtained for the surface tension of undiluted normal blood serum is 52 dynes per centimeter at 20° and 48 dynes at 37°C The result at the higher temperature agrees moderately well with the mean value found by Morgan and Woodward (7), but it is known that the method by which their results were calculated gives values which are too low The average of all of their values at 37° is 45.5 while the average of all of our results is 46.2 However, their work represents a great forward step, as it is of a much higher degree of accuracy than that of any of the other earlier investigators Almost all of the values obtained by others are of an altogether different order of magnitude, since they are from 10 to 20 dynes too high

The worthlessness of much of the work is due to an altogether incorrect assumption, which is that if only relative values are desired no attention needs to be paid to the theory of the method used Thus the method in which the number of drops given by a certain volume of the liquid with a certain tip (drop number method) is supposed to be inversely proportional to the surface tension, is practically useless,

as is the ring method as commonly used. The determination of capillary height is the most accurate of all known methods for determination of the surface tension of pure liquids which are not viscous. When used with blood serum, and with most other biological liquids, it has, however, the disadvantage that the film at the surface often prevents the attainment of capillary-hydrostatic equilibrium and it may affect the angle of contact. The bubble pressure method suffers from the disadvantage that as usually applied the experimental procedure does not agree with the conditions set up by the theory of the method. A much greater disadvantage is that it is difficult almost impossible to hold the bubble at its maximum pressure for the considerable time necessary to establish equilibrium between the interior of the serum and its surface.

A careful consideration of all of the available methods seemed to indicate that the best results for such liquids may be obtained with the special modification of the drop weight method described later in the paper. The drop weight method avoids difficulties which relate to the angle of contact and the measurements are much less affected by the surface viscosity than when the capillary height method is used.

#### THE DROP WEIGHT APPARATUS FOR THE DETERMINATION OF SURFACE TENSION OF BLOOD SERUM AND OTHER BIOLOGICAL LIQUIDS

The apparatus used for the determination of the surface tension of blood serum is a modified form of the drop weight apparatus of Harkins and Brown (13), which resembles in general design that of Morgan (14).

The apparatus (fig. 1) consists essentially of an inverted capillary U-tube, suspended in a metal box (*B*), which is provided with glass windows in front of and back of the tip from which the drop of serum is suspended. One limb of this inverted U-tube dips into a reservoir (*S*) of the serum. The other limb ends in the tip which is held by a stopper of glass or metal in the weighing bottle (*V*), in which the drops are collected as they fall.

Each drop is pulled over, until it attains the maximum size consistent with stability, by the action of suction applied through the metal tube (*A*), and the drop is held at this maximum extension for several minutes before it is allowed to fall.

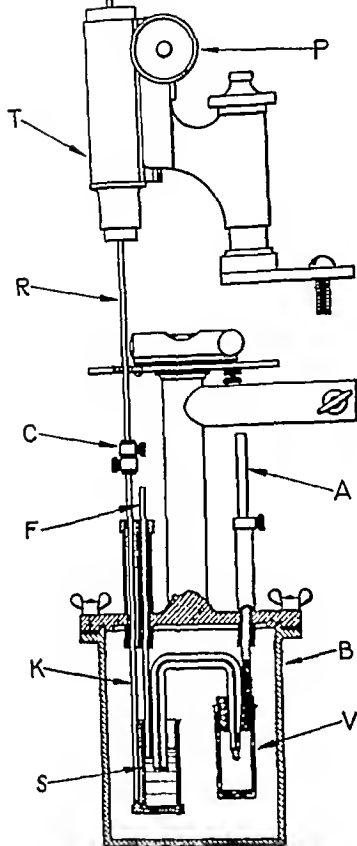


FIG 1 APPARATUS FOR THE DETERMINATION OF THE SURFACE TENSION OF BLOOD SERUM AND OTHER BIOLOGICAL LIQUIDS BY THE DROP WEIGHT METHOD

V, weighing bottle under tip for collecting drops, S, reservoir for serum, KCR, rod for controlling the speed of drop formation, P, rack and pinion for adjusting height of rod R, F, tube for saturating serum with alveolar air, A, suction tube, and B, box for keeping out water of thermostat. The tip of pyrex glass is circular and shows no flaws in its edge under a magnification of  $20\times$ . The bottom of the tip is first ground off plane. Then a circular piece of glass of the same diameter as the tip is sealed over it by means of de Khotinsky cement. The sides of the tip are then ground down to the required diameter in a precision lathe. The tip is rotated in one direction, and it is ground by a high speed, very fine, disc of carborundum, which rotates in the opposite direction and is finally given a high polish by means of rouge. When the cement is melted a tip ground properly in this way is found to be circular and perfectly sharp. Eight different diameters of the tip are measured to 0.0001 cm each.

as is the ring method as commonly used. The determination of the capillary height is the most accurate of all known methods for the determination of the surface tension of pure liquids which are not too viscous. When used with blood serum, and with most other biological liquids, it has, however, the disadvantage that the film at the surface often prevents the attainment of capillary-hydrostatic equilibrium and it may affect the angle of contact. The bubble pressure method suffers from the disadvantage that as usually applied the experimental procedure does not agree with the conditions set up by the theory of the method. A much greater disadvantage is that it is difficult or almost impossible to hold the bubble at its maximum pressure for the considerable time necessary to establish equilibrium between the interior of the serum and its surface.

A careful consideration of all of the available methods seemed to indicate that the best results for such liquids may be obtained by the special modification of the drop weight method described later in the paper. The drop weight method avoids difficulties which relate to the angle of contact and the measurements are much less affected by the surface viscosity than when the capillary height method is used.

#### THE DROP WEIGHT APPARATUS FOR THE DETERMINATION OF SURFACE TENSION OF BLOOD SERUM AND OTHER BIOLOGICAL LIQUIDS

The apparatus used for the determination of the surface tension of blood serum is a modified form of the drop weight apparatus of Harkins and Brown (13), which resembles in general design that of Morgan (14).

The apparatus (fig. 1) consists essentially of an inverted capillary U-tube, suspended in a metal box (*B*), which is provided with glass windows in front of and back of the tip from which the drop of serum is suspended. One limb of this inverted U-tube dips into a reservoir (*S*) of the serum. The other limb ends in the tip which is held by a stopper of glass or metal in the weighing bottle (*V*), in which the drops are collected as they fall.

Each drop is pulled over, until it attains the maximum size consistent with stability, by the action of suction applied through the metal tube (*A*), and the drop is held at this maximum extension for seven minutes before it is allowed to fall.

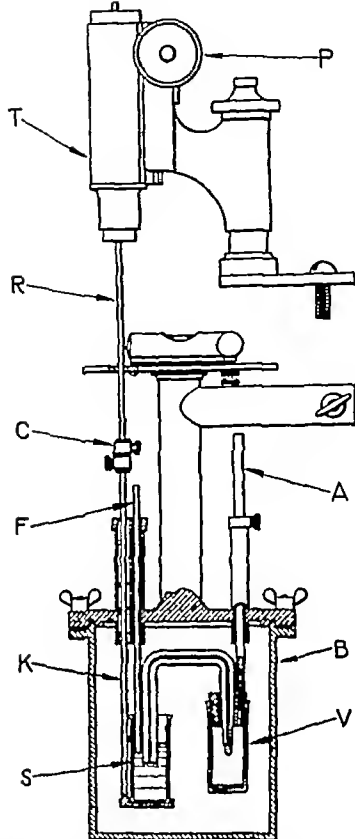


FIG 1 APPARATUS FOR THE DETERMINATION OF THE SURFACE TENSION OF BLOOD SERUM AND OTHER BIOLOGICAL LIQUIDS BY THE DROP WEIGHT METHOD

V, weighing bottle under tip for collecting drops, S, reservoir for serum, KCR, rod for controlling the speed of drop formation, P, rack and pinion for adjusting height of rod R, F, tube for saturating serum with alveolar air, A, suction tube, and B, box for keeping out water of thermostat. The tip of pyrex glass is circular and shows no flaws in its edge under a magnification of  $20\times$ . The bottom of the tip is first ground off plane. Then a circular piece of glass of the same diameter as the tip is sealed over it by means of de Khotinsky cement. The sides of the tip are then ground down to the required diameter in a precision lathe. The tip is rotated in one direction, and it is ground by a high speed, very fine, disc of carborundum, which rotates in the opposite direction and is finally given a high polish by means of rouge. When the cement is melted a tip ground properly in this way is found to be circular and perfectly sharp. Eight different diameters of the tip are measured to  $0.0001$  cm each.

The glass tube ( $F$ ) is used for the aeration of the serum with alveol air, as described in the section on aeration, or with another gas

During a determination the metal box ( $B$ ) is kept entirely immersed in the water of a thermostat at the proper temperature  $\pm 0.01^\circ$

It is extremely important that the serum in ( $S$ ) be adjusted to the level requisite to hold the drop at exactly full extension for the seven minute period. This is done by means of the rod ( $R$ ), which controls the level of the reservoir ( $S$ ). For the work described in the present paper this level was adjusted by hand, and the delicate control of the drop was effected by properly varying the suction applied at ( $A$ )

However, the difficulty of controlling the drop in this way led to the method of control described in the next paragraph

#### CONTROL OF THE DROP AND VARIATION OF THE DROP WEIGHT WITH TIME

Biological liquids contain in general surface-active substances, and the surface whose tension is being measured must be kept fully formed sufficiently long to allow equilibrium between the surface and the interior of the liquid to be attained. With aqueous solutions of decylic acid the minimum period of full extension is more than thirty minutes. With blood serum the surface tension falls for several minutes, and rises again if the surface becomes older than about seven minutes. This actual or apparent increase may be due to the formation of a solid film which does not represent a true condition of equilibrium with the interior of the liquid

A delicate adjustment of the pressure in the drop, which determines its extension, is obtained by suspending the rod ( $R$ ) from the bar ( $C$ ) which is raised and lowered by a ratchet and pinion. For this purpose a microscope stand with a coarse and fine adjustment is ideal

The "full extension of the drop" can be determined only by trial. The telescope of a cathetometer is levelled and the height of the telescope is so adjusted that the horizontal cross hair and the bottom of the drop at full extension appear coincident. The level of the serum in ( $S$ ) is so adjusted that the bottom of the drop will remain at this level for the desired period (of seven minutes). Several trials are necessary, even with the experienced worker. A common error is to consider that the level of full extension lies higher than is actually the case. To avoid this the level of the cross hair is lowered slightly

and an endeavor is made to adjust the level of the bottle (*S*) in such a way that the drop will not fall. If this is possible, the preceding level was too high.

An error in the opposite direction is apt to arise due to the decrease of the surface tension as the surface active substance diffuses into the surface from the body of the liquid. The length of the drop at "full extension" for the higher surface tension is greater than that for the lowest, or equilibrium tension. This makes it necessary, if a seven minute period is to be allowed, to determine the full extension at the end of this period.

TABLE I  
*Comparison of the weights of drop held at almost full extension for seven minutes and those held for a shorter period*

| Patient | Time           | Drop weight | Time           | Drop weight |
|---------|----------------|-------------|----------------|-------------|
|         | <i>minutes</i> |             | <i>minutes</i> |             |
| D       | $\frac{1}{2}$  | 0.06260     | 7              | 0.05393     |
| F       | $1\frac{1}{2}$ | 0.06070     | 7              | 0.05520     |
| G       |                |             | 7              | 0.05440     |
| S       | 1              | 0.05510     | 7              | 0.04770     |
| S       |                |             | 7              | 0.04780     |
| S       | 1              | 0.05860     | 7              | 0.04932     |

The tip used in the work in this and later tables on patients D to K inclusive has a diameter of 0.5944 cm. The tip used on all other patients has a diameter of 0.5000 cm.

It should be kept in mind that what is called full extension of the drop does not correspond to the greatest length which the drop may have, but to the greatest length which it may have without becoming unstable. Almost immediately after the drop becomes unstable, the neck of the drop narrows with great rapidity, that is, the drop pinches off by a lateral movement of its upper surface (fig. 2).

It was found that the use of a period of about seven minutes gave the minimum drop weight. If held longer than seven minutes, the drop weight seems to rise again, but this rise is probably due to the formation of a solid film. Thus, a sample which gave a drop weight of 0.05742 for a set of drops each held for a period of from 4 to 8 minutes, gave a drop weight of 0.05790 for a drop held twenty-one minutes. Table I shows a comparison between drops held seven

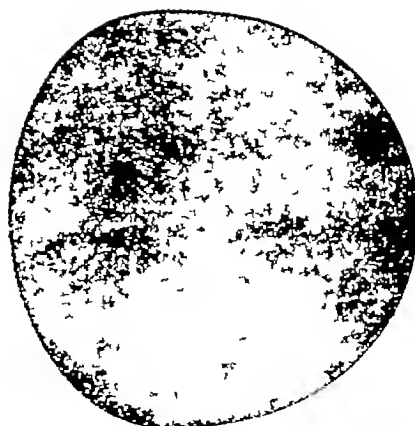
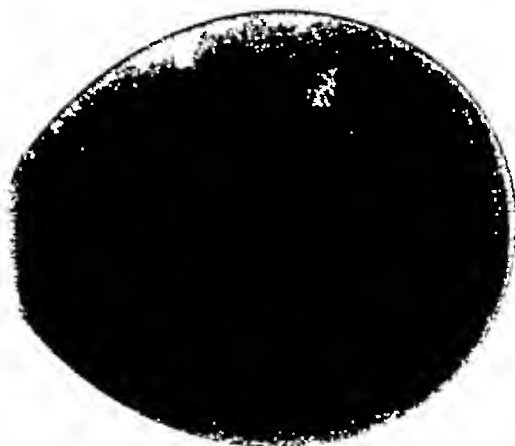
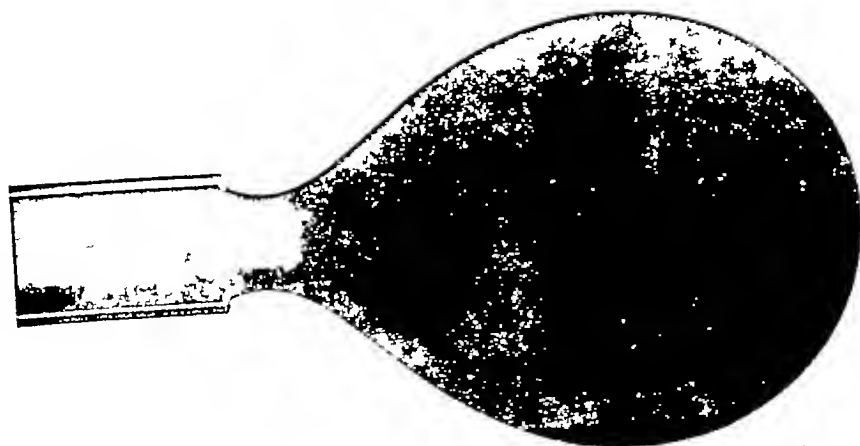
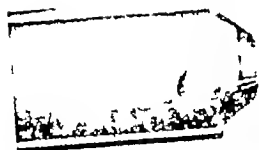
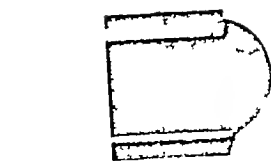


FIG 2, A

FIG 2, B

FIG 2, C

FIG 2A, B, AND C SHOWS THREE STEPS IN THE DETACHMENT OF A DROP

It will be noted that a part of the drop remains hanging on the tip. The way in which the drop detaches is dependent upon the rapidity with which it forms and falls, so for an accuracy of 0.1 per cent the drop must be caused to detach itself as slowly as possible. The control rod RCF (fig 1) enables this to be done so accurately that it is possible with pure liquids to reduce the error to about 0.05 per cent. The corrections are valid only for drops which detach slowly.

minutes and those held a shorter time. Since the time used to obtain a single drop is seven minutes, it was considered advisable to use only 2 to 3 drops per determination. It may be noted that Morgan and Woodward used only a single drop.

#### CALCULATION OF THE SURFACE TENSION

The surface tension ( $\gamma$ ) is not, as is often assumed, proportional to the weight of the drop when a single tip is used, but it may be calculated very easily from the following equation

$$\gamma = \frac{mg}{R} \times F$$

in which  $m$  is the weight in grams of the drop which falls (as weighed

TABLE 2  
*Drop-weight surface tension corrections*

(Factor for multiplication =  $F$ ) Based on the value 72.75 as the surface tension of water at 20°C.  $R$  is the radius of the tip.

| $\frac{V}{R^3}$ | $F$     | $\frac{V}{R^3}$ | $F$     |
|-----------------|---------|-----------------|---------|
| ( $\infty$ )    | 0.159   | 2.637           | 0.26224 |
| 5000.0          | 0.172   | 2.3414          | 0.26350 |
| 250.0           | 0.198   | 2.0929          | 0.26452 |
| 58.1            | 0.215   | 1.8839          | 0.26522 |
| 24.6            | 0.22561 | 1.7062          | 0.26562 |
| 17.1            | 0.23051 | 1.5545          | 0.26566 |
| 13.28           | 0.23522 | 1.4235          | 0.26544 |
| 10.29           | 0.23976 | 1.3096          | 0.26495 |
| 8.190           | 0.24398 | 1.2109          | 0.26407 |
| 6.662           | 0.24786 | 1.124           | 0.26324 |
| 5.522           | 0.25135 | 1.048           | 0.2617  |
| 4.653           | 0.25419 | 0.980           | 0.2602  |
| 3.975           | 0.25661 | 0.912           | 0.2585  |
| 3.433           | 0.25874 | 0.865           | 0.2570  |
| 2.995           | 0.26065 |                 |         |

in the gas or vapor in which it was suspended),  $g$  is the gravitational attraction, and  $R$  is the radius of the circular face of the tip.

The values of  $F$  have been determined by Harkins and Brown, and are given in table 2, in which  $V$  represents the volume of the drop which falls, and  $V = \frac{m}{\rho}$  in which  $\rho$  is the density of the liquid

## METHOD AND RESULTS

A large part of the work consisted in evolving a method which would give constant results. The data which were obtained by the procedure finally chosen will be discussed in the next section. The preliminary steps in the method are listed below.

TABLE 3A

*The surface tension of the serum falls to a more or less constant value in the first few hours after procuring the blood*

| Patient | Time after procuring blood | Drop weight |
|---------|----------------------------|-------------|
|         | <i>minutes</i>             |             |
| M       | 80                         | 0.05035     |
|         | 150                        | 0.04988     |
|         | 180                        | 0.04920     |
| N       | 75                         | 0.05147     |
|         | 105                        | 0.04942     |
|         | 210                        | 0.04957     |
| O       | 240                        | 0.05170     |
|         | 270                        | 0.05145     |
| P       | 75                         | 0.04865     |
|         | 105                        | 0.04808     |
|         | 240                        | 0.04832     |
| R       | 165                        | 0.05210     |
|         | 405                        | 0.04775     |
|         | 435                        | 0.04760     |
| S       | 465                        | 0.04770     |
|         | 510                        | 0.04780     |

Note. The centrifuging was done about twenty minutes before the first determination recorded in each case, the blood being kept sealed until the centrifuging. In cases N and O no centrifuging was necessary.

*1 Procuring the blood.* About 10 to 20 cc. of blood is taken from the median cubital vein of either arm by means of a hypodermic syringe. The syringe must be dry since water, ether, and similar substances cause hemolysis and there is a possibility that hemoglobin in solution may affect some of the measurements involved.

2 *Sealing the tube* If the determinations cannot be made immediately, the blood should be sealed in a tube which it almost fills, so as to be in contact with as little air as possible. The tube may be stoppered with a paraffined cotton plug but is preferably sealed by the use of a minute flame. If the latter method is used, the tube should be drawn out to a fine point at one end before introducing the blood. Then only a slight heating will finish the seal. It is well not

TABLE 3B

*The surface tension of samples of serum kept for varying periods of time (sealed, at 5°C) before centrifuging*

| Patient | First determination drop weight | Interval | Second determination drop weight | Interval | Third determination |
|---------|---------------------------------|----------|----------------------------------|----------|---------------------|
|         |                                 | days     |                                  | days     |                     |
| M       | 0.04920                         | 1        | 0.04875                          | 2        | 0.04788             |
| N       | 0.04942                         | 1        | 0.04900                          | 3        | 0.05000             |
| O       | 0.05145                         | 4        | 0.05235                          | 3        | 0.04860             |
| P       | 0.04808                         | 1        | 0.04860                          |          |                     |
| Q       | 0.04810                         | 1        | 0.04848                          |          |                     |
| S       | 0.04770                         | 1        | 0.04932                          |          |                     |

TABLE 3C

*The lowering of the surface tension of serum on standing for several days*

| Patient | First determination drop weight | Interval | Second determination drop weight |
|---------|---------------------------------|----------|----------------------------------|
|         |                                 | days     |                                  |
| D       | 0.06509                         | 4        | 0.06260                          |
| F       | 0.05742                         | 2        | 0.05510                          |

to have the blood come quite to the end of the tube in this case, as it is difficult to seal glass covered with blood.

3 *The interval between the procuring of the blood and the observations* The interval between the procuring of the blood and the determination has been observed to be of the greatest import. In general, other things being equal, several types of variation with time occur. These will be discussed in order.

a If the blood is centrifuged immediately, and the determinations

made at once, it is observed that the surface tension decreases rapidly within the first few hours to a more or less constant value. This is demonstrated by table 3A which gives all the data that concern this phenomenon.

*b* If the blood is kept sealed, without centrifuging for several days, there is no definite change in the surface tension. This is determined by sealing several different portions of the same sample of blood in separate tubes, and opening, centrifuging, and finding the surface tension of the serum from one tube each day. Table 3B shows that the surface tension of a serum may increase or decrease or vary irregularly with the time the separate samples are kept.

*c* After being centrifuged, the surface tension of serum decreases gradually over a period of days as is seen in table 3C.

*d* If blood is kept for several days in a sealed tube and then centrifuged, it exhibits a rapid decrease in surface tension to a more or less constant value during the first few hours, which decrease is usually not as large as that observed in (*a*). This is shown in table 4.

*4 Cleaning the apparatus* The apparatus is cleaned by sucking water through it immediately after the completion of the day's work. The serum should not be allowed to dry on the apparatus, nor should it be cleaned with alcohol, ether, or cleaning mixture as these reagents precipitate the proteins in the serum and clog the capillary.

*5 Determinations of density and refractive index* The density was determined by the Hammerschlag method (15), and the refractive index was determined by the use of a Bausch and Lomb Abbé Refractometer.

*6 The aeration of the serum* Alveolar air is blown through a long coiled tube immersed in the thermostat and into the serum through the tube mentioned above. This tube is first carefully flushed out with alveolar air from the experimenter's lungs before immersing the lower end of the short tube in the serum. When the tube is well flushed out, the distal end is dipped into the serum and alveolar air bubbled through for some time. This is done to secure equilibrium between the serum and a gas of constant composition approaching as nearly as possible that in the lungs. Saturation with alveolar air causes varying effects depending on the previous condition of the serum. In general the surface tension is lowered as is seen in table 5.

TABLE 4

*The surface tension of blood serum kept at 5 C for several days*

Note that the tension falls to a more or less constant value in the first few hours after centrifuging

| Patient | Age of blood before centrifuging | Drop weight |
|---------|----------------------------------|-------------|
|         | <i>days</i>                      |             |
| M       | 4                                | 0 05130     |
|         | 4                                | 0 04960     |
|         | 4                                | 0 05052     |
|         | 4                                | 0 04950     |
| C       | 2                                | 0 04870     |
|         | 2                                | 0 04950     |
|         | 2                                | 0 05000     |
| M       | 1                                | 0 04830     |
|         | 1                                | 0 04925     |
|         | 3                                | 0 04787     |
|         | 3                                | 0 04816     |
|         | 3                                | 0 04763     |
| N       | 1                                | 0 05040     |
|         | 1                                | 0 04820     |
|         | 1                                | 0 04975     |
|         | 1                                | 0 04896     |
|         | 4                                | 0 04920     |
|         | 4                                | 0 05080     |
| O       | 4                                | 0 05513     |
|         | 4                                | 0 05485     |
|         | 4                                | 0 05300     |
|         | 4                                | 0 05285     |
|         | 4                                | 0 05235     |
|         | 7                                | 0 05395     |
|         | 7                                | 0 05105     |
| P       | 7                                | 0 04860     |
|         |                                  |             |
| P       | 1                                | 0 04915     |
|         | 1                                | 0 04860     |
| Q       | 1                                | 0 04852     |
|         | 1                                | 0 04845     |

\* For each sample the determinations are listed in order and were performed at one-half hour intervals.

## THE TEMPERATURE COEFFICIENT

Due to the fact that many of the determinations were made at the Presbyterian Hospital where there is no thermostat, it was necessary to determine the temperature coefficient of the surface tension of serum. These observations were made on the blood of Patient S, an almost normal subject. The results obtained at four temperatures from 10° to 37°C are exhibited in table 6.

TABLE 5  
*Effect of aeration with alveolar air upon the surface tension of the serum*  
Patient D

| Drop weight before aeration | Drop weight after aeration |
|-----------------------------|----------------------------|
| 0.06787                     | 0.06509                    |
| 0.06743                     | 0.06574                    |

TABLE 6  
*The temperature coefficient of serum surface tension*

| Temperature | Drop weight | Surface tension |
|-------------|-------------|-----------------|
| °C          |             |                 |
| 10          | 0.05340     | 54.26           |
| 20          | 0.04932     | 50.35           |
| 30          | 0.04645     | 47.58           |
| 37          | 0.04505     | 46.22           |

## PATHOLOGICAL VARIATIONS

The results are not of such a nature that any definite conclusions can be drawn as to a lowering or raising of the surface tension as a result of any pathological condition. Many of the cases had several pathological factors, any one of which might have altered the surface tension. Furthermore the work of Brinkman (8) on oxalated plasma and of Zunz (9) on serum indicates that the surface tension is higher in women than in men. Until these results are confirmed or disproved, it would be best to allow a margin of error in comparing the serum surface tension of men and women.

The results in relation to the pathology of the different patients are shown in table 7. In most cases the corresponding density and

refractive index are given. It is apparent that no two sets of these results run perfectly parallel. The surface tension is observed to be

TABLE 7

*The surface tension of blood serum as related to the pathology of the patients from whom the blood was obtained*

Temperature = 20°C

| Patient | Diagnosis  | Surface tension | Refractive index | Density† |
|---------|--|-----------------|------------------|----------|
| I       | Normal   | 53.55           |                  | 56       |
| J       | Normal   | 51.01           |                  | 2        |
| K       | Normal   | 51.02           |                  | 2        |
| L       | Diabetes   | 53.93           |                  | 0        |
| M       | General paresis, given typhoid treatment for syphilis few days before, collapsed after spinal puncture elsewhere, male, 36 years   | 50.41           | 20               | 42       |
| M       | A week later   | 49.71           | 38               | 44       |
| C       | Chronic interstitial nephritis, arterial hypertension, 7 successful pregnancies, bad vertigo, albumin, epistaxis, stroke in November 1925, good renal efficiency, female, 40 years | 50.43           | 8                | 20       |
| N       | Parenchymatous nephritis, hemoglobin of 28 per cent, red blood cells of 2 500 000, female, 40 years  | 50.52           | 3                | 25       |
| O       | Sciatica, chronic sacroiliac arthritis, chronic tonsillitis, female, 42 years  | 51.77           | 16               | 33       |
| P       | Paroxysmal tachycardia of migraine, acute cold, male 40 years  | 49.37           | 15               | 32       |
| Q       | Trigone cystitis, cells in kidney urines, bad cold, colored male 45 years  | 49.37           | 27               | 53       |
| R       | Parenchymatous nephritis, myocarditis, fat, female 39 years  | 48.70           | 16               | 28       |
| S       | Bad case of influenza three weeks before Wasserman++++ 6 years ago, negative since, anemia, male, 48 years   | 49.57           | 0                | 8        |

\* For simplicity the refractive index tabulated above is the number of ten thousandths; the refractive index exceeds 1.390.

† Similarly, the densities are expressed as ten thousandths above 1.0222, the lowest value.

low in the cases just recovering from influenza, and in neither of the two cases suffering from parenchymatous nephritis, both of whom are women, is the surface tension quite as high as the average. This

latter result would tend to agree somewhat with the results of Clausen (10). But as Clausen gives no data in support of his conclusion, it is not known how much lowering of surface tension was obtained in parenchymatous nephritis. The high value of tension in the single diabetic case is interesting.

#### THE RING METHOD FOR THE DETERMINATION OF THE SURFACE TENSION OF BLOOD SERUM

The ring method has been used in an enormous number of the determinations of surface tension carried out with blood serum or solutions of blood serum in water. In nearly all cases the results obtained are almost valueless, since errors of 20 per cent are not uncommon. Such results are in general incorrect from even the relative standpoint, since the errors are highly variable.

*1 The calculation of the surface tension* The most common error in the use of the ring method is that made in the calculation of the surface tension. The surface tension is calculated from the pull exerted by the surface of the liquid on a circular ring (of radius  $R$ ) of circular wire (of radius  $r$ ). What is usually determined is the maximum pull ( $W$ ) on the ring as determined by a torsion balance, or better by the proper type of analytical or assay balance. This pull is obtained by allowing the horizontal ring to come into contact with the surface and then lowering the liquid or raising the balance until the film of liquid upheld by the ring is ruptured. It is customary to consider the maximum pull ( $P$ ) per centimeter of surface attached to the wire to be defined by the equation

$$P = \frac{Wg}{4\pi R} \quad (1)$$

It is obvious that the surface tension ( $\gamma$ ) is given by the identity

$$\gamma = P \frac{\gamma}{P} \quad \text{or} \quad \gamma = PF \quad (2)$$

In order that equation (2) may be used for the calculation of the surface tension of the liquid the values of the ratio  $\left(\frac{\gamma}{P} \text{ or } F\right)$  must be known. These values might have been calculated from theory, but

this has not as yet been done. The values of this ratio were determined by Harkins, Young and Cheng (16), and more carefully later by Harkins and Jordan (17). The values of this fraction as now known seem to be accurate to within 0.3 per cent, which gives considerable accuracy to the ring method, though it is still less accurate than the drop weight method under the best conditions (error 0.1 per cent).

The values of the correction factor  $F$  of the equation

$$\gamma = \frac{W_g}{4\pi R} F \quad (3)$$

are given in table 8. These correction factors are valid only if the ratio of the radius of the ring ( $R$ ) to that of the wire ( $r$ ) is 40, which is the ratio most commonly used.

TABLE 8

Values of the correction factor ( $F$ ) of the ring method (Harkins and Jordan) for a ring in which  $R = 0.6366$  cm,  $r = 0.01570$  cm and  $R/r = 40.55$

(This is the size of ring most generally used)

| $\frac{R^3}{V}$ | $\gamma/F$ |
|-----------------|------------|
| 0.43            | 0.988      |
| 0.5             | 0.973      |
| 0.6             | 0.954      |
| 0.7             | 0.938      |
| 0.8             | 0.925      |
| 0.9             | 0.914      |
| 1.0             | 0.905      |
| 1.1             | 0.898      |
| 1.2             | 0.892      |

The correction factor ( $F$ ) is a function of  $\frac{R^3}{V}$  in which  $V$  is the volume of liquid lifted by the pull ( $W$ ) of the ring above the level of the plane portion of the sufficiently large surface of the liquid, so

$$V = \frac{\pi g}{\rho} \quad (4)$$

in which  $\rho$  is the density of the liquid.

In order to determine the surface tension the value of the total

pull ( $W$ ) is first determined by the balance. From this ( $P$ ) is calculated by substituting the mean radius ( $R$ ) of the ring in equation (1), and the volume ( $V$ ) is calculated from equation (4). The value of  $\frac{R^3}{V}$  is then calculated, and from this the value of  $\frac{F}{\gamma}$

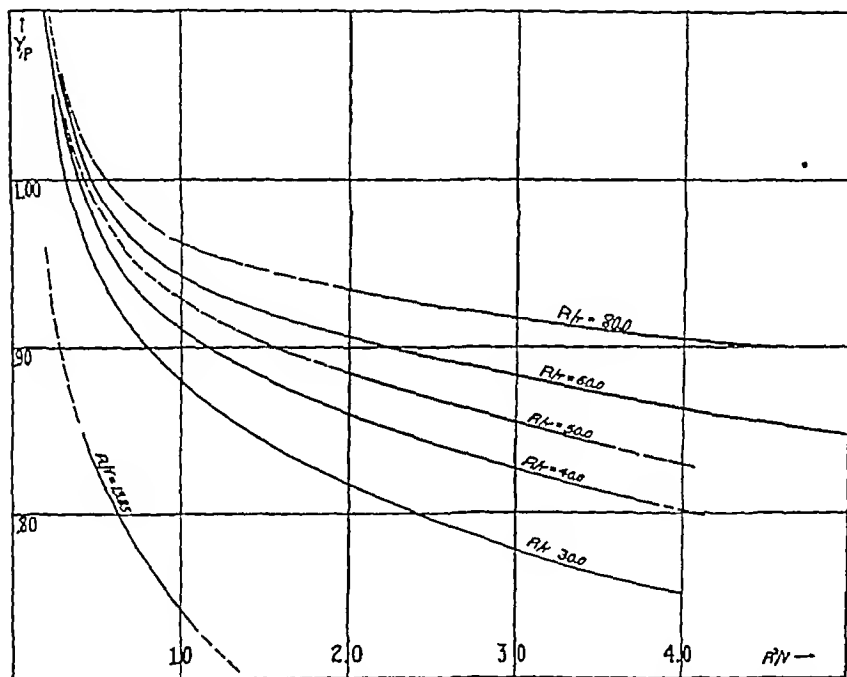


FIG. 3 CORRECTION CURVE FOR THE DETERMINATION OF THE SURFACE TENSION OF A LIQUID BY THE RING METHOD

Ordinates = values of  $F$ . Abscissae = values of  $R^3/V$ . The curves beginning at the bottom represent rings for which the values of the radius ( $R$ ) of the ring, divided by the radius ( $r$ ) of the wire are respectively 13.85, 30, 40, 50, 60, and 80.  $R$  is in centimeters.

$\gamma$ , surface tension,  $P$ ,  $Wg/4\pi R$ ,  $R$ , radius of ring,  $r$ , radius of wire,  $V$ , volume of liquid suspended by the upward pull of the ring.

is obtained by table 8. From this the surface tension  $\gamma$  is obtained by equation (3). Any simpler general method gives incorrect results.

The value of the correction factor ( $F$ ) varies also with the value of the ratio  $\frac{R}{r}$  as shown by figure 3. Table 8 gives the correction factors

which correspond with the fourth curve from the top. Any number of rings whose correction factors lie on this single curve may be used the only condition is that the radius of the ring shall be 40 times the radius of the wire, and that the ring shall be circular, plane, and, when suspended, perfectly horizontal.

*2 Area of the surface* The area of the surface of the liquid from which the ring is pulled should be large enough to meet the following condition just before the detachment of the ring there must be an annular ring of surface, completely around the ring of wire, in which the liquid surface is plane. The larger the ring of wire, the larger the total surface should be. The form of surface which is most economical of liquid is that of a circle, and for *small* rings this should be about 7 cm. in diameter.

*3 Surface tension and time* What is commonly designated as the surface tension of a liquid is the equilibrium value. It has been seen (page 267) that the time necessary to obtain equilibrium with undiluted blood serum is about seven minutes. With diluted serum this time may be more than an hour.

The surface tension of blood serum cannot be obtained by allowing the serum to stand in a vessel for the time given above (seven minutes for undiluted serum) and then pulling the ring from the surface. The correct procedure is as follows: blood serum is placed in the vessel and as quickly as possible the ring is placed in contact with its surface and at once the ring is lifted to just that distance above the serum, which at the end of the correct interval to give complete adsorption (seven minutes for undiluted serum), corresponds with the beginning of instability. In other words the surface must be fully extended initially and allowed to break without further extension at the end of the proper period.

In order that this may be done preliminary trials should be made to determine the proper extension, or different extensions may be applied and the pull ( $W$ ) plotted against the time ( $t$ ) of the rupture.

*4 Adjustment of the ring* It has been shown by Harkins and Jordan (17) that it is essential to adjust the ring in such a way that when it hangs from the balance its plane shall be horizontal. This has been done by obtaining the image of the ring in a plane horizontal mirror of gold or silver placed just below it.

## SUMMARY

1 The surface tension of normal blood serum is found to be about 52 dynes per centimeter at 20°C, and 48 dynes at 37°, and the surface tensions under certain pathological conditions have been determined. On the whole the values are lower than for normal serum. The purpose of the work of the paper has been to develop proper methods for the determination of the surface tension of biological liquids, rather than to secure a large number of data.

2 The worthlessness of the earlier work on the surface tension of blood serum is found to be due to (a) failure to obtain equilibrium conditions, or (b) improper methods of calculation.

3 An apparatus for the determination of the surface tension of biological liquids by the drop weight method has been developed, together with a new method for the control of the drop in such a way as to give accurate results.

4 The methods used in procuring the blood and in sealing up the serum are described.

5 The effects upon the surface tension of the time, before and after centrifuging, have been investigated.

6 Saturation of the serum with alveolar air at 20°C is found to lower the surface tension by about 2 dynes.

7 Correction tables for the calculation of the surface tension by (a) the drop weight method, and (b) the ring method, are given. The almost universal failure to use these corrections has caused earlier work to be of little value.

8 A method is given for the use of the ring method in the determination of the surface tension of biological liquids. The conclusion is reached that the drop weight method is somewhat superior to the ring method for such a purpose when both are properly used.

The writers wish to thank Drs Wilber E. Post and William A. Thomas for suggesting this problem and for advice concerning it.

## BIBLIOGRAPHY

- 1 Bugha, G., *Biochem. Ztschr.*, 1908, xi, 311. Veränderungen der Oberflächenspannung des Blutserums unter dem Einfluss von verschiedenen Elektrolyten.

- 2 Fillipi, E, *Lo sperimentale*, 1909, *lxiii*, 373 Su I Rapporti Tra I Mutamenti della Viscosita e della Tensione Superficiale del Sangue Vivente Sott L'Influenza di Vari Farmaci
- 3 Iscovesco, H, *Compt. rend Soc. d Biol*, 1911, *lxx*, 66 Études stalagmométriques. La Tension superficielle du sérum sanguin
- 4 Juhnke L A, *Interstate Med J*, 1911, *xviii*, 233 The Meistagmin Reaction
- 5 Fano G, and Maver, d. M, *Arch di fisiol*, 1907, *iv*, 165 Sulla tensione superficiale del siero di sangue
- 6 Marghini, Francescò, *Arch farm sper*, 1912, *xiii*, 53 Experimental Studies on the Surface Tension of Blood Serum
- 7 Morgan, J L R., and Woodward, H E, *J Am Chem Soc*, 1913, *xxxv*, 1249 The Weight of a Falling Drop and the Laws of Tate XI The Drop Weight and Surface Tension of Blood Serum
- 8 Brinkman, R., *Arch neerland physiol.*, 1922, *vii*, 258 Sur la tension superficielle du sang humain normal.
- 9 Zunz, M, and La Barre, J, *Bull Acad Roy Med. Belg*, 1924 *iv* 5th series, 74 Recherches sur la tension superficielle du plasma et du sérum humains à l'état normal et dans la syphilis
- 10 Clausen, S W, *Proc. Am Soc. Biol Chemists*, *J Biol Chem*, 1924, *lix* p xlv Studies in Parenchymatous Nephritis
- 11 DuNouy, P L, *J Exper Med*, 1925, *xli*, 663 Surface Tension of Serum XII A Technique for the Accurate Study of the Drop in Function of the Time See also other papers in the same journal
- 12 Marriott, McK., *Proc Inst Med Chicago*, 1925, *v*, 231 Application of Recent Studies on Nature of Nephritis
- 13 Harkins, W D, and Brown, F E, *J Am. Chem Soc*, 1916, *xxxviii* 246 A Simple Apparatus for the Accurate and Easy Determination of Surface Tension
- 14 Morgan J L R., *J Am Chem. Soc*, 1911, *xxxiii*, 349 The Weight of a Falling Drop and the Laws of Tate III An Apparatus for Rapid and Accurate Determination of the Weight of a Falling Drop of Liquid
- 15 Webster, R W, *Diagnostic Methods* Blakiston, 1923, 7th ed, p 458
- 16 Harkins, W D, Young, T F, and Cheng, L H, *Science*, 1926, *lxiv*, 333 The Ring Method for the Determination of Surface Tension
- 17 Harkins, W D, and Jordan, H F Unpublished results on Accurate Corrections for the Ring Method for the Determination of Surface Tension



# BLOOD VOLUME PRECEDING AND FOLLOWING SPLENECTOMY IN HEMOLYTIC ICTERUS AND SPLENIC ANEMIA<sup>1</sup>

BY HERBERT Z. GIFFIN AND GEORGE E. BROWN

WITH THE TECHNICAL ASSISTANCE OF GRACE M. ROTH

(From the Division of Medicine, The Mayo Clinic, Rochester)

(Received for publication December 26 1928)

Very little investigative work has been published concerning the relationship of the spleen to blood volume. Barcroft, Harris, Orshovats, and Weiss allot to the spleen a function that correlates well with its anatomic structure. They demonstrated that the spleen acts as a reservoir in the normal resting animal, and that blood is expressed into the general circulation as a result of stimulation to a need for oxygen. The presence of a storage function is further demonstrated by the observation that carboxyhemoglobin disappears less rapidly from the spleen than from the general circulation, indicating that hemoglobin is, under normal conditions, stored in the spleen. These workers demonstrated the greater susceptibility of the splenectomized animal to carbon monoxide intoxication. Cruickshank's experiments on the cat indicate that the capacity of the spleen is from 2 to 5 per cent of the blood volume when calculated by fluctuations in hemoglobin and in changes in the volume of the spleen.

Observations concerning the spleen and splenectomy in relation to blood volume in man have not been found in the literature. The studies carried out previously have dealt largely with variations in the concentration of the erythrocytes and the hemoglobin, the morphology, and the chemistry of the blood. We know that splenectomy is curative in certain diseases and markedly beneficial in others, and definitely untoward effects attributable to splenectomy have not been observed in man, although there has been some question as to whether a factor of safety is removed by this operation.

<sup>1</sup> Read by title at the meeting of the American Society for Clinical Investigation, Washington, D. C., April 28, 1928.

Our study was undertaken to determine (1) the blood volume and plasma volume in patients suffering from certain diseases associated with splenomegaly, (2) the effects of splenectomy on the blood and plasma volume, and (3) whether in certain forms of splenomegaly the anemia is apparent rather than actual. The cases selected for this study fall into four groups

|                                       | <i>number of cases</i> |
|---------------------------------------|------------------------|
| 1 Normal subjects                     | 74                     |
| 2 Primary splenomegaly without anemia |                        |
| Preceding splenectomy                 | 5                      |
| Following splenectomy                 | 4                      |
| 3 Hemolytic icterus                   |                        |
| Preceding splenectomy                 | 11                     |
| Following splenectomy                 | 9                      |
| 4 Splenic anemia                      |                        |
| Preceding splenectomy                 | 18                     |
| Following splenectomy                 | 7                      |

The dye method was used to determine the blood volume and plasma volume. Congo-red was employed and three to four minutes allowed for the mixing time. Determinations were made under resting conditions without breakfast (8).

It has been shown in anemia that with a decrease in the hemoglobin and cell volume there is usually an increase in plasma volume. This is doubtless a compensatory phenomenon designed to maintain an adequate volume of circulating blood. The increase of plasma varies in different forms of anemia, according to Rowntree and Brown, it is absent or slight in the anemia of glomerulonephritis with edema and in the anemia associated with myxedema and Addison's disease. In this connection we have employed the term "replacement index" to represent the ratio of the percentage increase in plasma to the percentage decrease in cell volume.

For example, if the plasma volume is 61 cc for each kilogram (normal 51.2 cc) there would be an increase of 9.8 cc (19 per cent). If the cell volume is 21 cc for each kilogram (normal 36.5 cc) there would be a decrease of 15.5 cc (42 per cent). The ratio is  $\frac{19}{42} = 0.45$ , the replacement index is 45. This indicates roughly that for every 100 cc loss in cell volume there is approximately an increase of 45 cc in plasma.

## NORMAL SUBJECTS

Dreyer and Ray have maintained that blood volume is a function of surface area rather than of body weight. We have, therefore, expressed our data according to both surface area and body weight and the statistical treatment of these data has shown a slightly higher correlation of volume to surface area than to body weight in normally built subjects.

The results obtained in seventy four normal adult subjects (forty-nine males and twenty five females) have been reported by Rowntree and Brown. The ages varied from seventeen to sixty-three years. The mean weight was 65.9 kgm. The mean value for the blood volume for each kilogram of body weight was 87.7 cc (with a range of from 70 to 100 cc in 98 per cent of the cases). The mean value for the plasma volume was 51.2 cc. (with a range of from 42 to 60 cc). The mean value for the cell volume was 36.5 cc for each kilogram. The mean value for the blood volume for each square meter of surface area was 3278 cc. For the plasma volume it was 1920 cc for each square meter of surface area. The mean value for circulating hemoglobin was 13.8 grams for each kilogram of body weight, or 508 grams for each square meter of surface area. The cells by hematocrit were 41 per cent<sup>2</sup> by the dry oxalate method, and the hemoglobin for each 100 cc was 15.7 grams. These values closely approximate those obtained by Keith, Rowntree and Geraghty in their original report based on eighteen subjects. Many of the normal subjects varied considerably from the accepted average weight for height and age, but could not be classified as underweight or obese. When a classification of this group was made on the basis of a 10 per cent variation above or below the average standard for height and age, the mean values for the blood volume and plasma volume were only slightly different.

## PRIMARY SPLENOMEGALY WITHOUT ANEMIA

Cases of primary splenomegaly without anemia, when all etiologic factors have been excluded, such as syphilis, malaria, hemolytic icterus,

<sup>2</sup> In subsequent work it has been found that hematocrit values obtained by the dry oxalate method should be corrected by the addition of 3.4 per cent. Therefore, 41 per cent, as used here, should read 44.4 per cent.

TABLE 1  
Splenomegaly without definite anemia  
blood volume preceding and following splenectomy

| Case           | Sex | Age | Date              | Weight<br>kgm | Surface areas<br>sq m | Hemoglobin<br>grams<br>in each<br>100 cc | Erythrocytes<br>mill<br>ions<br>in each<br>cu<br>mm | Cells by hematocrit<br>per<br>cent | Blood        |  |  | Plasma       |                                       |   | Cells        |                                       |   | Hemoglobin      |                              |                                | Weight of spleen<br>grams |
|----------------|-----|-----|-------------------|---------------|-----------------------|--|---|------------------------------------|--------------|--|--|--------------|---------------------------------------|---|--------------|---------------------------------------|---|-----------------|------------------------------|--------------------------------|---------------------------|
|                |     |     |                   |               |                       |  |   |                                    | Volume<br>cc | Cubic centimeters for<br>each kilogram | Cubic centimeters for<br>each square meter | Volume<br>cc | Cubic centimeter for<br>each kilogram | Cubic square meter for<br>each square meter | Volume<br>cc | Cubic centimeter for<br>each kilogram | Cubic centimeter for<br>each square meter | Volume<br>grams | Grams for each kilo-<br>gram | Grams for each square<br>meter |                           |
| 1              | M   | 49  | November 23, 1922 | 76            | 1 83                  | 13 9                                     | 4 66  | 42                                 | 7,480        | 98 4,090                               | 4,340                                      | 57           | 2,370                                 | 3,140                                       | 41           | 1,720                                 | 1,020                                     | 13 4557         |                              |                                |                           |
| 2*             | M   | 25  | May 25, 1923      | 60            | 1 74                  | 15 6                                     | 5 10  | 41                                 | 6,330        | 105 3,745                              | 3,740                                      | 62           | 2,210                                 | 2,590                                       | 43           | 1,535                                 | 987                                       | 13 1568         |                              |                                |                           |
| 3              | M   | 36  | February 22, 1925 | 55            | 1 65                  | 16 8                                     | 3 90  | 46                                 | 6,870        | 124 4,240                              | 3,710                                      | 67           | 2,290                                 | 3,160                                       | 57           | 1,950                                 | 1,154                                     | 20 9700         |                              |                                |                           |
| 4*             | M   | 43  | February 2, 1926  | 58            | 1 70                  | 15 4                                     | 5 24  | 45                                 | 6,290        | 108 3,700                              | 3,460                                      | 60           | 2,040                                 | 2,830                                       | 48           | 1,660                                 | 969                                       | 16 6,570        |                              |                                |                           |
|                |     |     | June 2, 1925      | 62            | 1 68                  | 15 0                                     | 3 82  | 38                                 | 5,520        | 89 3,260                               | 3,430                                      | 55           | 2,040                                 | 2,090                                       | 34           | 1,220                                 | 828                                       | 13 3,493        |                              |                                |                           |
|                |     |     | June 12, 1925     | 62            | 1 68                  | 15 0                                     | 3 93  | 36                                 | 5,310        | 85 3,150                               | 3,395                                      | 54           | 2,015                                 | 1,915                                       | 31           | 1,135                                 | 796                                       | 12 8,475        |                              |                                |                           |
| 5*             | M   | 42  | April 5, 1927     | 70            | 1 82                  | 11 0                                     | 4 57  | 49                                 | 7,390        | 105 4,040                              | 4,500                                      | 64           | 2,460                                 | 2,890                                       | 41           | 1,580                                 | 813                                       | 11 6,446        |                              |                                |                           |
|                |     |     | April 15, 1927    | 65            | 1 79                  | 11 0                                     | 4 15  | 34                                 | 5,845        | 90 3,265                               | 3,865                                      | 59           | 2,160                                 | 1,980                                       | 31           | 1,105                                 | 643                                       | 9 9,359         |                              |                                |                           |
| 6*             | F   | 26  | July 22, 1927     | 60            | 1 65                  | 15 4                                     | 3 88  | 43                                 | 6,390        | 106 3,740                              | 3,640                                      | 60           | 2,170                                 | 2,750                                       | 46           | 1,570                                 | 984                                       | 16 4,596        |                              |                                |                           |
| Average values |     |     |                   |               |                       |  |   |                                    |              |  |  |              |                                       |   |              |                                       |   |                 |                              |                                |                           |
| Preceding      |     |     |                   | 65 2          | 1 75                  | 14 2                                     | 4 68  | 41                                 | 0 6,602      | 101 3,767                              | 3,894                                      | 59 6         | 2,224                                 | 2,708                                       | 41           | 1,543                                 | 923                                       | 4 13            | 6526                         | 8                              |                           |
| Following      |     |     |                   | 60 5          | 1 69                  | 14 5                                     | 3 96  | 39 7                               | 6,103        | 101 3,598                              | 3,652                                      | 60 0         | 2,158                                 | 2,451                                       | 41           | 1,440                                 | 894                                       | 0 15            | 0 532                        | 5                              |                           |

\* Cases in which estimations were made following splenectomy In case 6 the estimation was not made preceding splenectomy, and in cases 1 and 3 estimations were made preceding splenectomy only

chronic infectious splenomegaly and various blood dyscrasias, have ordinarily been regarded as potential splenic anemia in the pre anemic stage, and the splenic enlargement has been the outstanding clinical feature

There were six cases in this group Three were studied both before and after splenectomy Two cases were studied preceding splenectomy and one case only after the operation The results of these studies are shown in table 1

*Comment* The blood volume preceding splenectomy was 101 cc and the plasma volume was 59.6 cc These values are high While the ratio of cell to plasma volume, as denoted by the hematocrit value of 41 per cent, was normal. This state has also been disclosed in cases of chronic passive congestion and in arteriovenous fistula, conditions in which a compensatory increase in the volume of circulating blood seems to be necessary for circulatory efficiency This leads to the possibility that in primary splenomegaly without anemia, the increased circulatory bed due to the splenomegaly and enlarged blood vessels necessitates a larger blood volume for circulatory needs

The comparison of the average values of the two groups of cases before splenectomy and after splenectomy does not show significant change in blood volume, plasma volume or in the percentage of cells by hematocrit In case 5 with an interval of nine days, there was a decrease of 20 per cent in total blood volume and of 3 per cent in plasma volume. Since the weight decreased 5 kgm, the changes in volume for each kilogram were not so marked This could be ascribed to operation and loss of blood The large blood volumes in the cases with long intervals, one year and one and a half years after splenectomy, are probably more significant, these patients have a large volume of normal blood, an unusual condition in subjects of normal build

1

#### HEMOLYTIC ICTERUS

The clinical features of hemolytic icterus are well known The fundamental process is abnormally active hemolysis and the pathologic basis is probably chiefly concerned with increased fragility of the erythrocytes and microcytosis (Giffin) In the diagnosis of hemolytic icterus care must be taken to exclude cirrhosis of the liver with

TABLE 2  
*Hemolytic icterus blood volume preceding and following splenectomy*

| Case | Sex | Age | Date               | Weight<br>kgm | Surface area<br>sq m | Hemoglobin<br>gm<br>each<br>100<br>cc | Erythrocytes<br>mill<br>ions<br>in each<br>cu<br>mm | Cells by hematocrit | Blood        |                                       |   | Plasma       |                                       |   | Cells        |                                       |   | Hemoglobin      |                              |                                | Weight of spleen<br>grams |
|------|-----|-----|--------------------|---------------|----------------------|---------------------------------------|---|---------------------|--------------|---------------------------------------|---|--------------|---------------------------------------|---|--------------|---------------------------------------|---|-----------------|------------------------------|--------------------------------|---------------------------|
|      |     |     |                    |               |                      |                                       |   |                     | Volume<br>cc | Cubic centimeter for<br>each kilogram | Cubic centimeter for<br>each square meter | Volume<br>cc | Cubic centimeter for<br>each kilogram | Cubic centimeter for<br>each square meter | Volume<br>cc | Cubic centimeter for<br>each kilogram | Cubic centimeter for<br>each square meter | Volume<br>grams | Grams for each kilo-<br>gram | Grams for each square<br>meter |                           |
| 1    | F   | 57  | May 21, 1925       | 45            | 1 45                 | 8 1                                   | 2 33  | 18                  | 4,480        | 100                                   | 3,090                                     | 3,680        | 82                                    | 2,540                                     | 800          | 18                                    | 550                                       | 363             | 8 0                          | 250                            |                           |
|      |     |     | June 2, 1925       | 45            | 1 45                 | 8 2                                   | 3 72  | 24                  | 3,510        | 77                                    | 2,420                                     | 2,660        | 59                                    | 1,840                                     | 850          | 19                                    | 580                                       | 288             | 6 4                          | 199                            | 800                       |
| 2    | M   | 31  | June 24, 1925      | 67            | 1 87                 | 12 4                                  | 3 54  | 28                  | 4,950        | 74                                    | 2,650                                     | 3,550        | 53                                    | 1,900                                     | 1,390        | 20                                    | 750                                       | 614             | 9 1                          | 1328                           |                           |
|      |     |     | July 4, 1925       | 62            | 1 80                 | 13 6                                  | 3 84  | 35                  | 6,920        | 109                                   | 3,840                                     | 4,500        | 71                                    | 2,500                                     | 2,420        | 40                                    | 1,340                                     | 941             | 15 0                         | 522                            | 1,450                     |
| 3    | F   | 35  | November 24, 1925  | 50            | 1 50                 | 8 6                                   | 3 60  | 23                  | 4,350        | 87                                    | 2,900                                     | 3,360        | 67                                    | 2,240                                     | 990          | 19                                    | 660                                       | 374             | 7 5                          | 249                            |                           |
|      |     |     | December 8, 1925   | 45            | 1 41                 | 12 5                                  | 4 16  | 33                  | 5,160        | 120                                   | 3,660                                     | 3,460        | 80                                    | 2,450                                     | 1,700        | 38                                    | 1,210                                     | 644             | 14 3                         | 457                            | 820                       |
| 4    | F   | 43  | November 24, 1925  | 61            | 1 70                 | 8 1                                   | 3 74  | 20                  | 4,240        | 70                                    | 2,490                                     | 3,390        | 55                                    | 1,995                                     | 850          | 14                                    | 495                                       | 343             | 5 6                          | 202                            |                           |
|      |     |     | December 8, 1925   | 54            | 1 61                 | 11 7                                  | 3 74  | 35                  | 5,360        | 99                                    | 3,330                                     | 2,950        | 54                                    | 1,830                                     | 2,410        | 44                                    | 1,500                                     | 627             | 11 6                         | 389                            | 945                       |
| 5*   | F   | 22  | September 15, 1925 | 52            | 1 46                 | 6 7                                   | 3 02  | 20                  | 4,320        | 83                                    | 2,940                                     | 3,450        | 66                                    | 2,350                                     | 870          | 17                                    | 590                                       | 289             | 5 6                          | 198                            | 1,640                     |
|      |     |     | May 24, 1926       | 20            | 0 83                 | 8 3                                   | 2 84  | 19                  | 3,060        | 153                                   | 3,690                                     | 2,470        | 123                                   | 2,975                                     | 590          | 30                                    | 715                                       | 254             | 12 0                         | 306                            |                           |
| 6    | F   | 6 5 | June 3, 1926       | 20            | 0 83                 | 12 1                                  | 3 80  | 30                  | 2,570        | 125                                   | 3,130                                     | 1,800        | 90                                    | 2,190                                     | 870          | 43                                    | 940                                       | 311             | 15 0                         | 375                            | 500                       |
|      |     |     | July 31, 1925      | 18            | 0 77                 | 8 9                                   | 3 69  | 24                  | 1,560        | 87                                    | 2,025                                     | 1,190        | 66                                    | 1,545                                     | 370          | 20                                    | 480                                       | 139             | 7 7                          | 180                            |                           |
| 7    | F   | 4   | August 14, 1925    | 18            | 0 77                 | 14 1                                  | 4 21  | 34                  | 1,580        | 87                                    | 2,100                                     | 1,050        | 58                                    | 1,400                                     | 530          | 29                                    | 700                                       | 223             | 13 0                         | 290                            | 203                       |
|      |     |     | July 27, 1926      | 23            | 1 00                 | 6 8                                   | 2 57  | 16                  | 2,050        | 89                                    | 2,050                                     | 1,720        | 75                                    | 1,720                                     | 330          | 14                                    | 330                                       | 139             | 6 0                          | 139                            |                           |
| 8    | M   | 9   | August 14, 1926    | 23            | 1 00                 | 12 9                                  | 3 88  | 30                  | 2,090        | 82                                    | 2,480                                     | 1,460        | 58                                    | 1,740                                     | 630          | 28                                    | 740                                       | 410             | 18 0                         | 410                            | 550                       |

|              |   |    |                  |    |      |      |      |      |      |       |       |       |       |       |       |       |       |       |         |          |
|--------------|---|----|------------------|----|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|----------|
| 9*           | F | 22 | January 13 1927  | 54 | 1 60 | 7 7  | 2 24 | 24   | 97   | 5,210 | 3 250 | 3 960 | 73    | 2 475 | 1,250 | 24    | 775   | 411   | 7 6257  |          |
| 10           | M | 14 | February 24 1926 | 32 | 1 16 | 10 3 | 3 65 | 20   | 83   | 2 670 | 2,300 | 2,140 | 67    | 1 860 | 530   | 17    | 440   | 275   | 8 6237  |          |
|              |   |    | March 11, 1926   | 31 | 1 16 | 11 2 | 4 24 | 30   | 109  | 3,370 | 2,900 | 2,360 | 76    | 2 000 | 1,010 | 32    | 900   | 377   | 12 0325 |          |
|              |   |    | May 12 1925      | 54 | 1 60 | 11 4 | 2 93 | 25   | 92   | 4 950 | 3 090 | 3,710 | 69    | 2 320 | 1 240 | 23    | 770   | 564   | 10 5353 |          |
| 11           | M | 27 | May 20 1925      | 50 | 1 52 | 10 2 | 3 93 | 33   | 123  | 6 190 | 4,020 | 4,140 | 82    | 2 630 | 2 050 | 40    | 1,390 | 631   | 12 0415 |          |
| Mean values† |   |    |                  | 42 | 71   | 38   | 86   | 3 10 | 22 3 | 3 795 | 93 22 | 791   | 2,932 | 73 1  | 2,200 | 836 4 | 21 7  | 597 7 | 359     | 8 1247 7 |

\* In cases 5 and 9 blood volume was not estimated after splenectomy

† Mean values estimated on data preceding splenectomy only

secondary hemolytic characteristics Splenectomy is a curative measure Following operation the jaundice disappears, the excessive hemolysis ceases and, in some instances at least, the erythrocytes become less fragile As Moynihan stated "The spleen, if not the exclusive cause or seat of the disease, exerts the profoundest influence on its pathogeny "

*Preceding splenectomy* Eleven cases of hemolytic icterus were studied (table 2) The body build in this group was subject to wide variation, none of the patients were overweight, but the younger patients were underweight Studies of the blood volume were made one or two days preceding operation, and from nine to seventeen days following operation Anemia was present in all of the cases The patient with a blood volume of 153 cc was the only one with a volume above the upper range of normal, she was an emaciated girl aged six and a half years, and according to surface area the blood volume was less than the normal mean According to surface area the mean plasma volume for the entire group was 2200 cc for each square meter, about 15 per cent higher than the normal mean

That anemia was actually present is shown by the amount of circulating hemoglobin The mean value for hemoglobin was 8.1 grams for each kilogram of body weight (normal 13.8 grams) The mean value for the cell volume was 21.7 cc for each kilogram (normal 36.5 cc), a decrease of 14.8 cc (45 per cent) The mean value for the plasma volume was 73.1 cc (normal 51.2 cc), an increase of 21.9 cc (43 per cent) The ratio was, therefore,  $\frac{1}{1.2} = 0.95$  This replacement index of 95 is high, almost twice that seen in primary and simple secondary anemia

The hemoglobin in grams and the blood volume when separately plotted against the weight of the spleen did not correlate The cell volume calculated for each kilogram of body weight plotted against the weight of the spleen did not show definite correlation There was a fairly high correlation of 0.87 between plasma volume and weight of the spleen

*Following splenectomy* Estimations were made from nine to seventeen days following splenectomy in nine of the eleven cases (table 2) The plasma volume according to body weight and surface area showed a smaller percentage variation since the body weight decreased

after operation The total hemoglobin volume increased in all but one case, the range of increase being from 57 to 327 grams, averaging

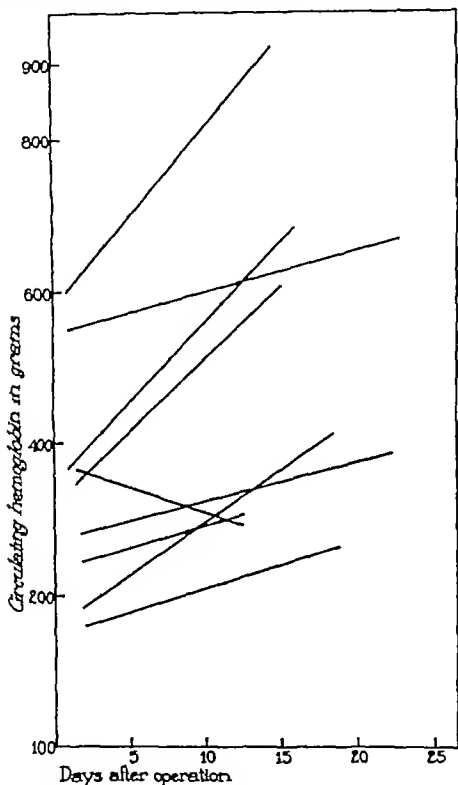


FIG 1 CHANGES IN THE CIRCULATING HEMOGLOBIN IN CASES FOLLOWING SPLENECTOMY FOR HEMOLYTIC JAUNDICE

183 grams (fig 1) The percentage of cells by hematocrit and the number of erythrocytes increased in every case, and the hemoglobin in grams for each 100 cc showed a significant increase in all but two cases

*Comment* The status of the blood volume in hemolytic icterus, according to mean values, is that of decreased cell volume with normal blood volume. The cell volumes and the hemoglobin volumes according to body weight and surface area were decreased, which is indicative of the existence of an actual rather than an apparent anemia. With the exception of one case, that of a child, the blood volume conformed closely to the normal range, which is 70 to 100 cc for each kilogram. The plasma volume, however, was greatly increased, the increase amounting to a mean of 21.9 cc, 43 per cent for each kilogram above the normal mean. The replacement index was high, 95, in other words, when compared to normal values the increase of plasma to replace the decrease in cells was almost complete. This indicates that in hemolytic icterus the replacement of the diminished cell volume by plasma is effective and adequate, much more so, in fact, than in primary anemia and simple secondary anemia without splenomegaly.

The increase in the blood volume and cell volume following splenectomy in cases of hemolytic icterus is most striking and correlates well with the clinical improvement following operation. In every case but one the cell volume increased significantly within three weeks and the actual volume of hemoglobin showed comparable changes, the increase averaging 183 grams. In case 2 (table 2) the increase in the blood volume was more than a third of the preoperative value, and the actual increase of hemoglobin was 327 grams in ten days, or over 30 grams a day. This increase of hemoglobin represents only the demonstrable increase of pigment and does not include the amount necessary to compensate for that which is normally destroyed. Figures for the normal amount of hemoglobin formed daily in man are not available. Whipple, Robschiet and Hooper have shown that in the dog after unit hemorrhages, the amount of hemoglobin regenerated depends largely on the type of diet. In one experiment with beef heart diet, 43 grams of hemoglobin were formed daily over a seven-day period.

The rate of recovery from anemia after splenectomy is not correctly shown in the concentration values for hemoglobin and erythrocytes. For example, in case 2 (table 2) following operation there was an increase of about 9 per cent in the grams of hemoglobin in each 100 cc and a questionable increase in the number of erythrocytes. The

increase in the blood volume, however, was 1970 cc (40 per cent) half of which was cells. The increase in the actual volume of hemoglobin was 327 grams, or about 50 per cent. The plasma volume increased 940 cc while the cell volume increased 1030 cc, that is, cells increased in greater proportion than plasma, thus affecting the ratio of cells to plasma.

Hooper, Robscheit and Whipple have shown that in the recovery from experimental anemia, the increased plasma volume caused by the anemia gradually disappears with the regeneration of cells. In the recovery from anemia in hemolytic icterus, the plasma volume decreased in five cases with the increase of cells, and increased in four cases.

The status of the blood volume as indicated by the mean value in a series of eleven cases of hemolytic icterus preceding splenectomy is that of decreased cells with normal volume. The anemia of hemolytic icterus is an actual rather than an apparent anemia and as the plasma volume increases to cause a slightly high blood volume a small amount of dilution occurs. The blood volume is maintained within a normal range since the replacement of cell volume by plasma is almost complete, as shown by a replacement index of 95. Following splenectomy there is an immediate rapid increase in the cell volume and hemoglobin volume which is not adequately demonstrated by the ordinary clinical estimations for determining the concentration of hemoglobin and erythrocytes.

#### SPLENIC ANEMIA

Splenic anemia is a useful term to indicate a poorly defined clinical syndrome rather than a disease entity, and the diagnosis is chiefly made by exclusion. Cases included in this group have two features in common: primary splenomegaly, and a secondary type of anemia. Cases of questionable diagnosis are always present in a group of cases classified as splenic anemia, and the difficulties of arriving at conclusions concerning observations on the blood volume are correspondingly increased, especially in those cases in which secondary hepatic cirrhosis is present.

*Preceding splenectomy.* Estimations of blood volume were made preceding splenectomy in eighteen cases classified as splenic anemia,

TABLE 3  
Splenic anemia blood volume preceding\* and following splenectomy

| Case | Sex | Age | Date               | Weight<br>kgm | Surface area<br>sq m | Hemoglobin<br>grams<br>in each<br>100 cc | Erythrocytes<br>mil<br>lions<br>in each<br>cu<br>mm | Cells by hematocrit<br>per<br>cent | Blood        |  |   | Plasma       |  |   | Cells        |  |   | Hemoglobin      |                              |                                | Weight of spleen<br>grams |
|------|-----|-----|--------------------|---------------|----------------------|--|---|------------------------------------|--------------|--|---|--------------|--|---|--------------|--|---|-----------------|------------------------------|--------------------------------|---------------------------|
|      |     |     |                    |               |                      |  |   |                                    | Volume<br>cc | Cubic centimeters for<br>each kilogram | Cubic square meter<br>for each square meter | Volume<br>cc | Cubic centimeters for<br>each kilogram | Cubic square meter<br>for each square meter | Volume<br>cc | Cubic centimeters for<br>each kilogram | Cubic square meter<br>for each square meter | Volume<br>grams | Grams for each kilo-<br>gram | Grams for each square<br>meter |                           |
| 1    | F   | 13  | November 2, 1923   | 53            | 1.70                 | 11.8                                     | 3,502.9   | 5,780                              | 109          | 3,610.4                                | 100.77                                      | 2,560.1      | 680                                    | 31  | 982.681      | 13.0                                   | 400   | 13.0            | 400                          |                                |                           |
| 2†   | M   | 41  | December 11, 1926  | 74            | 1.96                 | 8.3                                      | 4,343.0   | 8,300                              | 112          | 4,230.5                                | 800.78                                      | 2,960.2      | 500                                    | 34  | 1,288.688    | 9.0                                    | 352   | 9.0             | 352                          |                                | 300                       |
| 3    | F   | 31  | July 17, 1925      | 69            | 1.77                 | 7.6                                      | 3,502.6   | 4,630                              | 67           | 2,620.3                                | 430.50                                      | 1,940.1      | 200                                    | 17  | 677.338      | 5.0                                    | 190   | 5.0             | 190                          |                                | 400                       |
| 4†   | M   | 47  | January 27, 1926   | 80            | 1.93                 | 9.2                                      | 3,332.6   | 8,510                              | 107          | 4,410.6                                | 320.79                                      | 3,270.2      | 190                                    | 27  | 1,135.783    | 9.7                                    | 405   | 9.7             | 405                          |                                |                           |
|      |     |     | February 20, 1926  | 70            | 1.82                 | 11.2                                     | 3,002.5   | 7,280                              | 104          | 4,000.5                                | 450.77                                      | 2,950.1      | 830                                    | 26  | 1,010.815    | 11.6                                   | 448   | 11.6            | 448                          |                                | 1,460                     |
| 5    | F   | 23  | June 23, 1926      | 44            | 1.30                 | 8.5                                      | 3,433.2   | 4,720                              | 107          | 3,630.3                                | 210.73                                      | 2,470.1      | 510                                    | 34  | 1,160.401    | 9.0                                    | 308   | 9.0             | 308                          |                                | 700                       |
|      |     |     | January 21, 1927   | 40            | 1.31                 | 9.5                                      | 3,702.6   | 3,955                              | 98           | 3,020.2                                | 930.73                                      | 2,235.1      | 025                                    | 25  | 788.375      | 9.3                                    | 286   | 9.3             | 286                          |                                |                           |
| 6†   | M   | 30  | October 14, 1926   | 87            | 2.02                 | 10.9                                     | 3,962.9   | 7,460                              | 86           | 3,690.5                                | 300.61                                      | 2,630.2      | 160                                    | 24  | 1,040.804    | 9.2                                    | 398   | 9.2             | 398                          |                                |                           |
|      |     |     | November 4, 1926   | 72            | 1.92                 | 10.7                                     | 3,703.0   | 7,345                              | 102          | 3,845.5                                | 140.71                                      | 2,680.2      | 205                                    | 30  | 1,160.786    | 10.9                                   | 410   | 10.9            | 410                          |                                | 1,440                     |
| 7†   | M   | 35  | May 31, 1927       | 72            | 1.87                 | 7.9                                      | 4,323.0   | 7,350                              | 102          | 3,910.5                                | 140.71                                      | 2,750.2      | 210                                    | 31  | 1,181.580    | 8.0                                    | 310   | 8.0             | 310                          |                                | 1,160                     |
| 8†   | F   | 28  | July 21, 1927      | 62            | 1.65                 | 11.6                                     | 4,293.4   | 6,380                              | 103          | 3,870.4                                | 210.68                                      | 2,550.2      | 170                                    | 35  | 1,321.741    | 12.0                                   | 450   | 12.0            | 450                          |                                |                           |
|      |     |     | August 12, 1927    | 57            | 1.59                 | 12.1                                     | 4,323.5   | 5,780                              | 101          | 3,635.3                                | 710.65                                      | 2,330.2      | 070                                    | 36  | 1,380.699    | 12.0                                   | 439   | 12.0            | 439                          |                                | 1,140                     |
| 9    | F   | 29  | September 4, 1927  | 49            | 1.46                 | 7.6                                      | 3,182.5   | 4,750                              | 97           | 3,250.3                                | 560.73                                      | 2,440.1      | 190                                    | 24  | 815.362      | 7.3                                    | 247   | 7.3             | 247                          |                                |                           |
|      |     |     | September 19, 1927 | 45            | 1.42                 | 6.6                                      |   | 4,570                              | 101          | 3,220.3                                | 330.74                                      | 2,345.1      | 240                                    | 27  | 885.302      | 6.7                                    | 213   | 6.7             | 213                          |                                | 1,000                     |
| 10   | F   | 34  | June 3, 1927       | 59            | 1.65                 | 12.8                                     | 4,042.2   | 6,400                              | 108          | 3,850.3                                | 710.63                                      | 2,240.2      | 690                                    | 45  | 1,670.819    | 14.0                                   | 511   | 14.0            | 511                          |                                |                           |
|      |     |     | June 20, 1927      | 53            | 1.60                 | 12.6                                     | 3,423.7   | 5,665                              | 106          | 3,545.3                                | 565.67                                      | 2,225.2      | 100                                    | 40  | 1,400.714    | 13.4                                   | 446   | 13.4            | 446                          |                                | 986                       |

|                 |   |    |                    |    |        |      |      |    |        |       |        |       |       |        |       |     |       |       |       |       |       |
|-----------------|---|----|--------------------|----|--------|------|------|----|--------|-------|--------|-------|-------|--------|-------|-----|-------|-------|-------|-------|-------|
| 11              | F | 40 | May 10, 1927       | 56 | 1 62   | 11 1 | 3 69 | 37 | 5,860  | 104   | 3,620  | 3 690 | 66    | 2,270  | 2,170 | 381 | 340   | 650   | 12 0  | 401   | 1,000 |
| 12              | F | 42 | September 14, 1927 | 53 | 1 59   | 12 0 | 3 96 | 32 | 6,010  | 113   | 3 780  | 4,090 | 77    | 2,570  | 1,920 | 361 | 207   | 721   | 13 0  | 453   | 800   |
| 13              | M | 49 | September 22, 1927 | 85 | 2 00   | 5 6  | 2 71 | 21 | 7,120  | 84    | 3,560  | 5,620 | 66    | 2,800  | 1 500 | 18  | 750   | 399   | 5 0   | 197   |       |
| 14              | M | 55 | February 28, 1928  | 70 | 1 76   | 7 5  | 2 79 | 23 | 6 500  | 93    | 3 700  | 5,000 | 71    | 2,840  | 1 500 | 22  | 860   | 486   | 7 0   | 276   |       |
| 15              | F | 18 | March 15, 1928     | 63 | 1 69   | 9 3  | 3 42 | 26 | 5 530  | 88    | 3,270  | 4,090 | 65    | 2,420  | 1,440 | 23  | 850   | 514   | 8 2   | 304   | 1,480 |
| 16              | F | 55 | December 8, 1926   | 60 | 1 65   | 10 0 | 3 86 | 31 | 5 930  | 99    | 3 590  | 4 090 | 68    | 2,480  | 1,840 | 301 | 115   | 593   | 9 9   | 359   | 1,800 |
| 17              | F | 59 | March 26, 1925     | 54 | 1 49   | 14 1 | 3 62 | 33 | 4,030  | 75    | 2,710  | 2,700 | 50    | 1 810  | 1,330 | 25  | 900   | 568   | 10 5  | 381   |       |
| 18              | M | 26 | June 21, 1927      | 51 | 1 49   | 8 1  | 3 41 | 26 | 5 750  | 112   | 3 860  | 4,250 | 83    | 2,840  | 1,500 | 301 | 1,006 | 466   | 9 0   | 312   |       |
|                 |   |    | October 22, 1925   | 73 | 1 92   | 11 2 | 3 68 | 32 | 5 760  | 79    | 3,000  | 3 920 | 53    | 2 040  | 1,840 | 25  | 958   | 645   | 8 8   | 335   | 1,170 |
| Mean values†    |   |    |                    | 64 | 2 1 72 | 9 8  | 3 70 | 30 | 36,194 | 5 97  | 53,600 | 4 888 | 68    | 62 512 | 1,855 | 301 | 078   | 594   | 4 9 7 | 355 6 |       |
| Average values‡ |   |    |                    | 57 | 1 1 62 | 10 3 | 3 59 | 29 | 45,732 | 0 100 | 03,505 | 4,031 | 70 32 | 455 1  | 701   | 301 | 068   | 600 7 | 10 3  | 363 7 |       |

\* In cases for which only one row of values is given, estimations were made preceding splenectomy only

† Ranti's disease.

‡ Mean values estimated on data preceding splenectomy only

§ Average values estimated on data following splenectomy only (cases 4, 5, 6, 8, 9, 10 and 14)

TABLE 3  
Splenic anemia blood volume preceding\* and following splenectomy

| Case | Sex | Age | Date               | Weight<br>kgm | Surface area<br>sq m | Hemoglobin<br>grams<br>in each<br>100 cc | Erythrocytes<br>mil<br>lions<br>in each<br>cu<br>mm | Cells by hematocrit | Blood        |  |   | Plasma       |  |   | Cells        |  |   | Hemoglobin      |                              |                                | grams | Weight of spleen |
|------|-----|-----|--------------------|---------------|----------------------|--|---|---------------------|--------------|--|---|--------------|--|---|--------------|--|---|-----------------|------------------------------|--------------------------------|-------|------------------|
|      |     |     |                    |               |                      |  |   |                     | Volume<br>cc | Cubic centimeters for<br>each kilogram | Cubic square meter for<br>each square meter | Volume<br>cc | Cubic centimeters for<br>each kilogram | Cubic square meter for<br>each square meter | Volume<br>cc | Cubic centimeters for<br>each kilogram | Cubic square meter for<br>each square meter | Volume<br>grams | Grams for each kilo-<br>gram | Grams for each square<br>meter |       |                  |
| 1    | F   | 43  | November 2, 1923   | 53            | 1 70                 | 11 8                                     | 3 50  | 29                  | 5,780        | 109                                    | 3,610                                       | 4,100        | 77                                     | 2,560                                       | 1,680        | 31                                     | 982   | 681             | 13 0                         | 400                            |       |                  |
| 2†   | M   | 41  | December 11, 1926  | 74            | 1 96                 | 8 3                                      | 4 34  | 30                  | 8,300        | 112                                    | 4,230                                       | 5,800        | 78                                     | 2,960                                       | 2,500        | 34                                     | 1,288                                       | 688             | 9 0                          | 352                            |       | 300              |
| 3    | F   | 31  | July 17, 1925      | 69            | 1 77                 | 7 6                                      | 3 50  | 26                  | 4,630        | 67                                     | 2,620                                       | 3,430        | 50                                     | 1,940                                       | 1,200        | 17                                     | 677   | 338             | 5 0                          | 190                            |       | 400              |
| 4†   | M   | 47  | January 27, 1926   | 80            | 1 93                 | 9 2                                      | 3 33  | 26                  | 8,510        | 107                                    | 4,410                                       | 6,320        | 79                                     | 3,270                                       | 2,190        | 27                                     | 1,135                                       | 783             | 9 7                          | 405                            |       |                  |
|      |     |     | February 20, 1926  | 70            | 1 82                 | 11 2                                     | 3 00  | 25                  | 7,280        | 104                                    | 4,000                                       | 5,450        | 77                                     | 2,950                                       | 1,830        | 26                                     | 1,010                                       | 815             | 11 6                         | 448                            |       | 1,460            |
| 5    | F   | 23  | June 23, 1926      | 44            | 1 30                 | 8 5                                      | 3 43  | 32                  | 4,720        | 107                                    | 3,630                                       | 3,210        | 73                                     | 2,470                                       | 1,510        | 34                                     | 1,160                                       | 401             | 9 0                          | 308                            |       | 700              |
|      |     |     | January 21, 1927   | 40            | 1 31                 | 9 5                                      | 3 70  | 26                  | 3,955        | 98                                     | 3,020                                       | 2,930        | 73                                     | 2,235                                       | 1,025        | 25                                     | 788   | 375             | 9 3                          | 286                            |       |                  |
| 6†   | M   | 30  | October 14, 1926   | 87            | 2 02                 | 10 9                                     | 3 96  | 29                  | 7,460        | 86                                     | 3,690                                       | 5,300        | 61                                     | 2,630                                       | 2,160        | 24                                     | 1,040                                       | 804             | 9 2                          | 398                            |       |                  |
|      |     |     | November 4, 1926   | 72            | 1 92                 | 10 7                                     | 3 70  | 30                  | 7,345        | 102                                    | 3,845                                       | 5,140        | 71                                     | 2,680                                       | 2,205        | 30                                     | 1,160                                       | 786             | 10 9                         | 410                            |       | 1,440            |
| 7†   | M   | 35  | May 31, 1927       | 72            | 1 87                 | 7 9                                      | 4 32  | 30                  | 7,350        | 102                                    | 3,910                                       | 5,140        | 71                                     | 2,750                                       | 2,210        | 31                                     | 1,181                                       | 580             | 8 0                          | 310                            |       | 1,160            |
|      |     |     | July 21, 1927      | 62            | 1 65                 | 11 6                                     | 4 29  | 34                  | 6,380        | 103                                    | 3,870                                       | 4,210        | 68                                     | 2,550                                       | 2,170        | 35                                     | 1,321                                       | 741             | 12 0                         | 450                            |       |                  |
| 8†   | F   | 28  | August 12, 1927    | 57            | 1 59                 | 12 1                                     | 4 32  | 35                  | 5,780        | 101                                    | 3,635                                       | 3,710        | 65                                     | 2,330                                       | 2,070        | 36                                     | 1,380                                       | 699             | 12 0                         | 439                            |       | 1,140            |
|      |     |     | September 4, 1927  | 49            | 1 46                 | 7 6                                      | 3 18  | 25                  | 4,750        | 97                                     | 3,250                                       | 3,560        | 73                                     | 2,440                                       | 1,190        | 24                                     | 815   | 362             | 7 3                          | 247                            |       |                  |
| 9    | F   | 29  | September 19, 1927 | 45            | 1 42                 | 6 6                                      |   | 27                  | 4,570        | 101                                    | 3,220                                       | 3,330        | 74                                     | 2,345                                       | 1,240        | 27                                     | 885   | 302             | 6 7                          | 213                            |       | 1,000            |
|      |     |     | June 3, 1927       | 59            | 1 65                 | 12 8                                     | 4 04  | 42                  | 6,400        | 108                                    | 3,850                                       | 3,710        | 63                                     | 2,240                                       | 2,690        | 45                                     | 1,670                                       | 819             | 14 0                         | 511                            |       |                  |
| 10   | I   | 34  | June 20, 1927      | 53            | 1 60                 | 12 6                                     | 3 42  | 37                  | 5,665        | 106                                    | 3,545                                       | 3,565        | 67                                     | 2,225                                       | 2,100        | 40                                     | 1,100                                       | 714             | 13 4                         | 446                            |       | 986              |

plasma volume and the weight of the spleen. It has been suggested that in the cases of enlarged spleen and a presumably active reticulo-endothelial system the dye is removed from the blood with abnormal rapidity. Information on this point has been obtained by the following experiments.

1. In two cases of hemolytic icterus volume determinations were made on the operating table just prior to removal of the spleen. Twenty to thirty minutes after the injection of the dye, samples of blood were taken from the splenic artery, splenic vein and spleen pulp. It was found that the concentration of the dye in the three samples was exactly the same. These experiments seem to demon-

TABLE 4  
*Percentage of variation in the plasma volume within six minutes after injection of the dye*

| Case | Time after injection |           |           |           |
|------|----------------------|-----------|-----------|-----------|
|      | 2 minutes            | 3 minutes | 4 minutes | 6 minutes |
| 1    | 100                  | 100       | 100       | 100       |
| 2    |                      | 100       | 101       | 107       |
| 3    | 100                  | 100       | 101       | 102       |
| 4    | 100                  | 100       | 100       | 104       |
| 5    | 100                  | 99        | 100       | 96        |
| 6    | 100                  | 97        | 100       | 100       |

\* The two-minute determination is taken as 100 per cent.

strate that the dye is not abnormally removed by the cells of the spleen in hemolytic icterus.

2. The rate of disappearance of the dye from the blood was ascertained by determining the plasma volume at periods of two, three, four and six minutes in a large series of normal subjects and in six cases of splenic anemia (table 4), demonstrates that an increased or abnormal rate of disappearance of the dye was not present in the cases of splenic anemia. Moreover, dye was not excreted in the urine within one hour after injection, as has been demonstrated in certain cases of nephrosis.

The effect of anemia on the increase in plasma volume was considered. Only rarely in cases of secondary anemia were high plasma volumes found. In thirty cases of primary and secondary anemia there

seven of the Banti type (table 3). It will be noted in table 3 that in the seven cases of the Banti type there was approximately the same high blood volume as that in the cases without advanced hepatic cirrhosis

The body build of this group of patients was fairly uniform. None was obese or emaciated. The mean figure for body weight was 64.2 kgm.

*Following splenectomy* In seven of the cases observations were made both before and after splenectomy. Estimations of blood volume were made from two to three weeks after operation (table 3).

*Comment* The mean value for the blood volume according to the body weight and surface area is increased in splenic anemia approximately 10 per cent above the normal. This increase is largely due to plasma volume since the cell volume is reduced 18 per cent. The mean value for the plasma volume according to weight and area is approximately 30 per cent above normal. The plasma volume is much higher in splenic anemia than in the chronic secondary types of anemia and in pernicious anemia with comparable hemoglobin and cell values. This fact suggests the question whether the anemia is actual or whether dilution factors play a rôle causing apparent anemia. The circulating hemoglobin in grams for each kilogram preceding splenectomy was 9.7 grams, or for each square meter of surface area 355.6 grams compared with a normal of 13.8 grams and 508 grams, respectively. This would indicate that actual anemia exists which is diluted by the abnormally high plasma volume. The anemia is due both to dilution and to actual loss of hemoglobin. The plasma not only replaces the lost cell volume but increases to a point beyond the original blood volume. The replacement index is very high, as is demonstrated by the following calculation. A mean cell volume of 30 cc for each kilogram, compared with a normal of 36.5 cc, is 65 cc, 17.8 per cent, less than normal, the mean value for the plasma volume of 68.6 cc (normal 51.2 cc) is 17.4 cc, 34 per cent, above normal. The replacement index would then be 191 ( $\frac{36.5}{17.8} = 1.91$ ). The replacement index as demonstrated in sixteen cases of secondary anemia was 42 and in ten cases of pernicious anemia was 30.

The reason for the increase in plasma volume in cases of splenic anemia is not clear although there is a close correlation between the

plasma volume and the weight of the spleen. It has been suggested that in the cases of enlarged spleen and a presumably active reticulo-endothelial system the dye is removed from the blood with abnormal rapidity. Information on this point has been obtained by the following experiments.

1 In two cases of hemolytic icterus volume determinations were made on the operating table just prior to removal of the spleen. Twenty to thirty minutes after the injection of the dye, samples of blood were taken from the splenic artery, splenic vein and spleen pulp. It was found that the concentration of the dye in the three samples was exactly the same. These experiments seem to demon-

TABLE 4  
*Percentage of variation in the plasma volume within six minutes after injection of the dye*

| Case | Time after injection |           |           |           |
|------|----------------------|-----------|-----------|-----------|
|      | 2 minutes            | 3 minutes | 4 minutes | 6 minutes |
| 1    | 100                  | 100       | 100       | 100       |
| 2    |                      | 100       | 101       | 107       |
| 3    | 100                  | 100       | 101       | 102       |
| 4    | 100                  | 100       | 100       | 104       |
| 5    | 100                  | 99        | 100       | 96        |
| 6    | 100                  | 97        | 100       | 100       |

\* The two-minute determination is taken as 100 per cent

strate that the dye is not abnormally removed by the cells of the spleen in hemolytic icterus.

2 The rate of disappearance of the dye from the blood was ascertained by determining the plasma volume at periods of two, three, four and six minutes in a large series of normal subjects and in six cases of splenic anemia (table 4), demonstrates that an increased or abnormal rate of disappearance of the dye was not present in the cases of splenic anemia. Moreover, dye was not excreted in the urine within one hour after injection, as has been demonstrated in certain cases of nephrosis.

The effect of anemia on the increase in plasma volume was considered. Only rarely in cases of secondary anemia were high plasma volumes found. In thirty cases of primary and secondary anemia there

was a moderate increase in the relative volume of plasma with mean values of 60 and 58 cc, respectively. This would indicate that the anemia is probably not the sole factor in the production of the high plasma volume in splenic anemia. It was demonstrated in the series of cases reported in the first portion of this paper that cases of splenomegaly without anemia also show a high plasma volume. It is possible, then, that a high plasma volume may be one of the fundamental changes in cases of splenic anemia, and that it may precede the development of anemia.

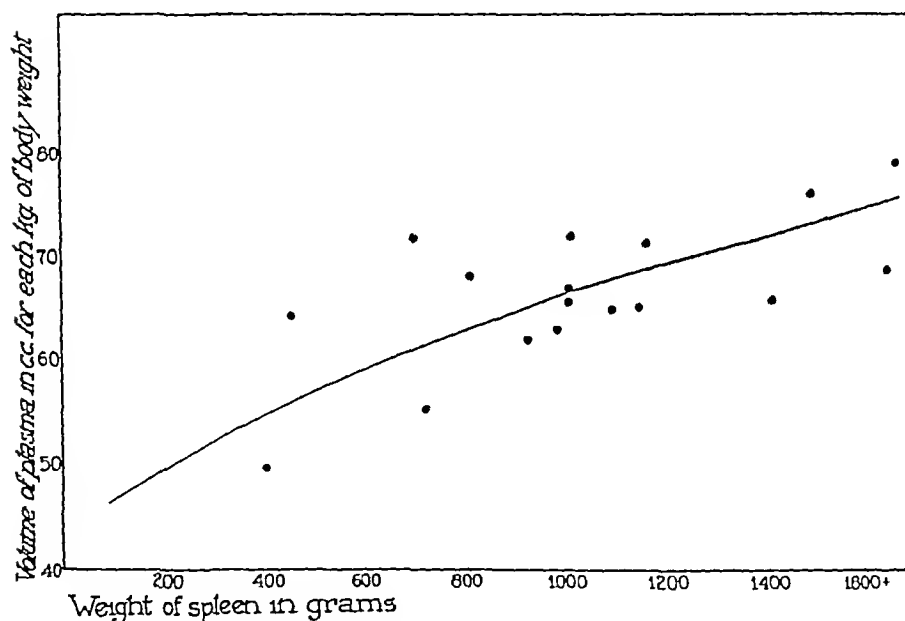


FIG 2 PLASMA VOLUME AND WEIGHT OF SPLEEN IN SPLENIC ANEMIA

Following splenectomy a decrease in the total volume of the circulating blood occurred in every case. This decrease was slight in one instance but definite in all the others. The maximal decrease was 1230 cc and the average was 656 cc. The decrease in the total blood volume was more constant and marked than that occurring in the relative blood volume for each kilogram because of variations in body weight. On the basis of surface area a decrease occurred in all but one case. The plasma volume likewise decreased in every instance, with a range of decrease from 145 to 910 cc, the average decrease being

442 cc Slight increases and decreases occurred according to body weight. The cell volume decreased in five of the seven cases, but was significant in only three cases. In only two cases did the cell volume show an increase. The total amount of circulating hemoglobin in grams showed a decrease in five of the seven cases, averaging 50 grams.

The total blood volume for each kilogram of body weight was charted against the weight of the removed spleen. The curve shows a rough correlation between these factors. It is apparent that larger volumes of blood occurred in the cases in which the spleen was largest. A closer correlation is brought out when the plasma volume for each kilogram is plotted against the weight of the spleen. The curve approximates a straight line (fig 2). Between the cell volume and the weight of the spleen definite correlation could not be demonstrated.

The moderate reduction in the blood volume after splenectomy in cases of splenic anemia would, at first thought, be attributed to loss of blood. The studies in these cases were made two and three weeks following operation, this interval would ordinarily be regarded as sufficient for adequate blood regeneration. There is great variation in the amount of blood lost at operation, in some cases it is negligible and in others large. There does not seem to be any correlation between the amount lost at operation and the decrease in blood volume. Likewise, the drop in the volume of blood after operation consists largely of plasma, indicating a readjustment following splenectomy. A relationship could not be established between the reduction in blood volume and plasma volume and the weight of the spleen, in fact, the patient (case 5, table 3) with the smallest spleen, showed the greatest decrease in blood volume. Surprisingly small amounts of blood were recovered from the enlarged spleens, the amount ranged from 100 to 500 cc. Either the contraction of the spleen eliminates much of the blood before it is removed, or its actual capacity is reduced, possibly because of fibrosis. One would infer from these observations that loss of blood due to removal of the spleen, although it may affect the results to a certain degree, does not account for all of the reduction in volume of blood, nor could it account for the disproportionate loss in the plasma volume and the relatively high plasma volumes remaining after splenectomy.

Summarizing these results in connection with splenic anemia, it has

apparently been shown (1) the plasma volume, and therefore the total blood volume, is high according to body weight in spite of a decreased volume of cells, (2) the increased plasma volume estimation is not due to an abnormal affinity of the cells of the spleen for the dye, to loss of the dye in the urine, or to an abnormally rapid rate of disappearance from the blood, (3) while the existence of anemia is usually a significant factor in the production of the high plasma volume, it is not the important or sole factor as it seems to be in cases of secondary anemia and pernicious anemia, (4) the enlarged spleen is probably an additional factor in the increase of plasma volume and total blood volume, even in splenomegaly without anemia the plasma volume is slightly increased, and (5) a fairly high correlation exists between the weight of the spleen and the plasma volume for each kilogram

#### SUMMARY

The figures obtained in blood volume studies in seventy-four normal adult subjects reported elsewhere by Rowntree and Brown are approximately as follows for both sexes, mean value for the blood volume for each kilogram of body weight, 87.7 cc, plasma volume for each kilogram, 51.2 cc, cell volume, 36.5 cc, circulating hemoglobin for each kilogram of body weight, 13.8 grams, and for each square meter of surface area, 508 grams, hemoglobin for each 100 cc, 15.7 grams, and cells by hematocrit, 41 per cent. On the basis of each square meter of surface area the values are approximately as follows: volume of blood, 3278 cc, volume of plasma, 1920 cc, and volume of cells, 1358 cc.

In cases of primary splenomegaly without anemia the mean value for the blood volume was increased to 101 cc for each kilogram, the plasma volume was increased to 59.6 cc, and the ratio of the cells to plasma was normal. After splenectomy there was no significant change in the blood volume or in plasma volume, the values still remaining above normal.

In hemolytic icterus the blood volume was normal and the cell volume decreased, the replacement of the diminished cell volume by plasma was complete. After splenectomy there was an increase in blood volume and a very striking increase in the volume of cells and hemoglobin. These increases were much greater than were indicated

In splenic anemia blood volume was increased and cell volume decreased. An increase in the plasma volume was found which could not be accounted for solely on the basis of anemia. The anemia was due both to dilution and actual loss of hemoglobin. Following splenectomy a moderate decrease, which was largely in the plasma volume, occurred in the total blood volume, yet the increased plasma volume persisted. A high correlation has been demonstrated for the relative plasma volume and the weight of the removed spleen.

Most of these observations were made within three weeks of the time of operation. The number of observations made at longer intervals subsequent to splenectomy have been too few to warrant discussion.

#### BIBLIOGRAPHY

- 1 Barcroft, J, Harris, H A, Orshovats, D, and Weiss, R., *J Physiol*, 1925, lx, 443. A contribution to the physiology of the spleen.
- 2 Cruickshank, E W H, *J Physiol*, 1926, lxi, 455. On the output of haemoglobin and blood by the spleen.
- 3 Drever, G, and Ray, W. *Proc. Roy. Soc. Lond.*, 1910, Series B, lxxxi, 545. The blood volume of mammals as determined by experiments upon rabbits, guinea pigs and mice, and its relationship to the body weight and to the surface area expressed in a formula.
- 4 Giffin, H Z, *Surg., Gynec., and Obstet.*, 1917, xxv, 152. Hemolytic jaundice, a review of seventeen cases.
- 5 Hooper, C W, Robschert, F S, and Whipple, G H. *Am. J. Physiol.*, 1920, llii, 263. Blood regeneration following simple anemia. V. The influence of Blaud's pills and hemoglobin.
- 6 Keith, N M, Rowntree, L G, and Geraghty, J T, *Arch. Int. Med.*, 1915, xvi, 547. A method for the determination of plasma and blood volume.
- 7 Moynihan Berkeley, G A, *The spleen and some of its diseases*. Philadelphia, W B Saunders Co., 1921.
- 8 Rowntree, L G, and Brown, G E, *Blood volume in health and disease* (In press).
- 9 Whipple, G H, Robschert, F S, and Hooper, C W, *Am. J. Physiol.*, 1920, liii, 236. Blood regeneration following simple anemia. IV. Influences of meat, liver and various extractives alone or combined with standard diets.



PROCEEDINGS OF THE FIRST MEETING OF THE CENTRAL  
SOCIETY FOR CLINICAL RESEARCH HELD IN  
CHICAGO, ILL., NOVEMBER 23, 1928

*The Influence of Respiration on Venous Pressure* By O. O. MEYER, M.D. (by invitation), and Wm. S. MIDDLETON, M.D., Madison, Wis.

Movement of blood into the thorax is favored by the increased negative intrapleural pressure on inspiration. Accordingly venous pressure in the distal peripheral veins of the forearm falls on inspiration. With the initial expiration and the coincident fall of intrapleural negative pressure, peripheral venous pressure increases. Under ordinary circumstances in the normal individual these changes are too insignificant to warrant clinical attention. However, theoretical considerations stated develop a practical importance when changes of respiratory rhythm occur, as in the induction of anesthesia and Cheyne-Stokes respiration. The physical exertion, respiratory arrest and probable hypoxia increase of the induction stage of general anesthesia lead to show a fall of the venous pressure. The period of hyperpnea of Cheyne-Stokes respiration invariably induces a fall of venous pressure whereas an increase occurs after the apneic phase.

Led by these observations to seek a practical application as a dynamic test of right ventricular integrity, a series of studies was pursued relative to the pressure response to the Valsalva and to the Mueller tests. Briefly stated, the results confirm the consensus of opinion as to the rise of venous pressure in the Valsalva experiment, but refute the belief that the Mueller test leads to a fall of venous pressure. In both instances in normal subjects a rise in pressure whose level apparently bears a direct relation to the duration of the expiration and to the energy of the resisted respiratory effort. Obviously the results of the Valsalva experiment have a definite bearing on the circulatory failure at child birth and on the so-called "bed pan" deaths in cardiac cases.

Further developments of this attack led to the application of the Valsalva experiment to the various stages of cardiac incompetency. It is believed that more prompt and higher early elevations of the venous pressure may be anticipated in event of right ventricular weakness, but the resisted expiratory effort must be maintained under this circumstance. The ultimate level of venous pressure in decompensated individuals under the conditions of the Valsalva experiment therefore does not approach that noted in normal controls. Hence a great value attaches to the study of the venous pressure reaction of such cases in the Valsalva experiment.

*The Cardiodynamic Effects of Stenosis of the Aorta on the Pulmonary Circuit and Right Heart* By LOUIS N. KATZ, M.D., and MORTIMER L. SIEGEL, M.D. (by invitation), Cleveland, Ohio

An attempt was made in the present investigation to determine whether or not the left ventricle can counteract the effects of an impediment to the onward flow of blood and prevent back pressure effects in the pulmonary artery and right heart. A marked constriction was applied at the root of the aorta and its effect on the pressure pulses in the left auricle, pulmonary artery, and right ventricle was followed in optically recorded curves.

Obstruction of the coronary return flow to the right heart had little or no effect on the curves showing that changes produced were due to an increased coronary flow.

Our results indicate that stenosis of the root of the aorta leads to a damming back of blood in the pulmonary artery and right ventricle sufficient to raise the pressure within them. The left ventricle, left auricle, and pulmonary veins are apparently inadequate to accommodate the excess.

*Observations on the Rumpel-Leede Phenomenon* By JOHNSON MCGUIRE, M.D. (by invitation), Cincinnati, Ohio

The occurrence of petechial cutaneous hemorrhages following artificially induced venous stasis has been described in various diseases.

Hecht, in 1907, published an account of petechiae observed in children ill with scarlet fever. These hemorrhages developed when loose folds of skin were picked up and held lightly between the fingers.

During an epidemic of scarlet fever in 1911, Rumpel and Leede noted the appearance of similar hemorrhages following the application of a tourniquet. They came to the conclusion that the test was specific for scarlet fever.

Stephen and later Weissman studied skin hemorrhages in a fairly large series of miscellaneous cases. Using the cuff of an ordinary sphygmomanometer stasis was induced by raising the pressure to 70 mm. of Hg and maintaining this level for five minutes.

Petechial hemorrhages occurred regularly in subacute bacterial endocarditis, frequently in cases of hypertension, occasionally in rheumatic fever, scurvy, and acute nephritis, never in tuberculosis, staphylococcus infections, pernicious anemia, or diabetes.

Further studies have been made on patients with a variety of diseases and on a number of normal controls.

*Description of method* The result in groups of individuals suffering from 25 diseases are recorded in table 1.

The results in 50 normals in table 2.

An examination of the results in table 1, shows a markedly positive result with the tourniquet test in all cases of subacute bacterial endocarditis. Inconstant findings occurred in the other diseases.

In the hypertensive group Weissmann obtained an extremely high percentage of positive reactions, while this series shows a number of negatives with equally high blood pressures. The probable explanation of this discrepancy is the fact that practically every one will give a positive result when the pressure is maintained at 100 mm for five minutes. To produce what he considered appropriate stasis Weissmann used pressures just below the diastolic levels in the cases of hypertension.

Table 2 shows that approximately one third of normals gave slightly positive results.

*Summary and conclusions* The occurrence of petechiae following venous stasis has been studied in 25 diseases and in a group of normal controls. The results are given in two tables.

In the few cases of subacute bacterial endocarditis examined, the result was uniformly strongly positive and suggests the sign may prove of differential diagnostic value.

The frequency of a slightly positive reaction in normals suggests that only markedly positive results be considered significant.

*The Effects of Temporary Occlusion of the Coronary Arteries* By DON C. SUTTON, M.D. (by invitation), Chicago, Ill.

1 A method for the study of the effect of temporary occlusion of the coronary arteries in unanesthetized dogs has been developed.

2 Temporary occlusion of the coronary arteries in the dog produces pain which is abolished by removal of the left stellate ganglion, by destruction of the perivascular nerves, but not by severance of the vagi.

3 The visceral pericardium is not sensitive to pain.

*The Significance of Electrocardiograms Showing Left Axis Deviation with Inverted T I and Upright T III and Q R S of Normal Duration* By DREW LUTEN, M.D., and EDWARD GROVE, M.D. (by invitation), St. Louis, Mo.

Experimental evidence (Wilson and Herrmann) indicates that electrocardiograms of this type result from impairment of conductivity in the right limb of the A-V bundle. Two examples of supporting clinical evidence are presented. Our conception of the cause of this defect refers it to coronary disease.

Such records in our files (237 in number) were found to be confined almost exclusively to patients exhibiting either definite or presumptive arterial disease. Heart disease, when it occurred (56 per cent of cases), was almost invariably of types referable, wholly or in part, to coronary involvement.

The higher incidence of such defect in the right limb is readily explained by the coronary distribution to the two divisions of the A-V bundle (Gross).

*Emaciation, Anemia, Belony, Chronic Diarrhea and Malabsorption of Fat A Nutritional Disturbance in Adults which Resembles Celiac Disease and Sprue* By WILLIAM H. HOLMES, M.D. (by invitation), Chicago, Ill.

The article deals with a group of six cases in which emaciation, anemia, tetany

and chronic diarrhea were the dominant symptoms. In certain instances the emaciation amounted to a loss of 50 per cent of the usual body weight. The anemia resembled Addisonian anemia but differed in that there were no signs of postero lateral sclerosis or of an acidity. Subjective symptoms consisting of tingling and numbness of the extremities were due to tetany and disappeared immediately with restoration of a normal blood calcium. The emaciation and anemia appear to be due to malabsorption of food. Starches and fats are poorly utilized. A high protein diet was well tolerated and did not aggravate the tetany. A markedly dilated and redundant colon was present in all cases. Dietetic control and restoration of normal mineral balance resulted in a cure in two instances and improvement in the remainder.

*Chemical Studies of Patients with Non-Tropical Sprue* By PAUL STARR, M D ,  
Chicago, Ill

This report consists of (1) a detailed account of the results of diet, thyroid, parathyroid and calcium medication on the metabolic rate, weight, blood calcium and stools of a patient with non-tropical sprue observed from May 1926 to November 1928 (2) the chemical data of calcium balance metabolism experiments on a second patient on low fat, high protein diet, normal high fat diet, and normal low fat diet, covering twenty-four three day periods during five months observations, (3) the effect of gastric digestion on the blood calcium in normal patients with a few observations on patients with sprue, consisting of a tabulation of the change in the blood calcium during a fractional meal in twenty instances. The suggestions in regard to the calcium metabolism of sprue to be drawn from these studies are as follows (1) Chronic latent tetany is present in non-tropical sprue (2) it may become acute with extreme depression of the blood calcium, (3) negative calcium balance could not be detected on low fat diets, (4) negative calcium balance was found on a high fat diet during diarrhea, (5) treatment calculated to correct this is beneficial, (6) there is an associated depressed basal metabolic rate, and (7) benefit from raising it by thyroid administration, (8) there is a slight but definite depression of the blood calcium during the gastric digestion of a fractional test-meal, (9) this is not dependent upon acid secretion

*Hyperparathyroidism* By HAROLD BULGER, M D , HENRY H DIXON, M D  
(by invitation) and DAVID P BARR, M D , St Louis, Mo

We have studied a patient presenting multiple giant-cell bone tumors, hypotonicity of muscles, bilateral nephrolithiasis, hypercalcemia and abnormal tendency to excrete calcium in the urine and a parathyroid tumor.

The removal of the parathyroid tumor caused severe and almost fatal tetany, hypocalcemia, change in the size of the bone tumors and abnormal tendency to the retention of calcium.

The symptoms of this patient closely resemble many cases which are scattered

through the literature but which have never been sufficiently defined. It is suggested that these are cases of true hyperparathyroidism.

*The Instance of Peptic Ulcer as Determined Post Mortem* By A. J. MILLER, M.D.,  
Louisville, Ky.

Careful examination of the stomach and duodenum in patients over fifteen years of age coming to autopsy at the University Hospital in Omaha, Nebraska, over a period of two years revealed an instance of peptic ulcer of 18.8 per cent. There are 202 cases in the series. Thirty-eight of them had definite evidence of ulceration in the stomach or duodenum, five of these, or 13.1 per cent, were diagnosed clinically, thirty-three, or 86.9 per cent, were not recognized. Twenty-one, or 55.2 per cent, were entirely healed lesions, and of these, fourteen, or two-thirds of the healed lesions, were in the duodenum. Thirteen, or 34.3 per cent, were active lesions, and of these, three, or approximately one-third, were in the duodenum. In four, or 10.5 per cent, there were active and healed lesions, all of which were in the stomach.

The instance of peptic ulcer is much greater than commonly believed. Those in the duodenum heal much more frequently than those in the stomach.

*A Study of Pulmonary Lesions Among Children and Young Adults* By J. A. MYERS, M.D., Minneapolis, Minn.

From the routine examinations of more than six thousand children and young adults, those with definite evidence of pulmonary lesions have been followed over a period of several years. They are grouped according to location of lesions.

Primary tuberculous foci and their relation to the adult form of tuberculosis have been studied.

Different steps in the healing of lesions are presented to show how well pulmonary lesions in childhood often heal. In another group, different steps in the advancement of pulmonary lesions are presented. More than a hundred cases of pulmonary lesions among teen-age girls and boys are included.

*Studies of Pulmonary Gangrene* By I. PILOR, M.D. (by invitation), Chicago, Ill.

The purpose of these studies is to clarify a clinical condition in which the etiology has not been well understood. Bacteriologic studies in our own cases reveal in most instances fusiform bacilli, spirochetes always with other bacteria, particularly the pyogenic cocci. As these organisms appear to be identical with those found in the oral cavity it is evident that the infection is due to invasion of mouth organisms into the lower respiratory tract. For the development of lesions in the patient as well as in the experimental animal certain contributing factors are necessary. In patients aspiration of mouth secretions during anesthesia and foreign bodies are outstanding predisposing causes. In the rabbit material introduced intratracheally as a rule results in no change unless a foreign substance (lipiodal) is added. The Levaditi preparations of the lesions often reveal a

and chronic diarrhea were the dominant symptoms. In certain instances the emaciation amounted to a loss of 50 per cent of the usual body weight. The anemia resembled Addisonian anemia but differed in that there were no signs of postero-lateral sclerosis or of an acidity. Subjective symptoms consisting of tingling and numbness of the extremities were due to tetany and disappeared immediately with restoration of a normal blood calcium. The emaciation and anemia appear to be due to malabsorption of food. Starches and fats are poorly utilized. A high protein diet was well tolerated and did not aggravate the tetany. A markedly dilated and redundant colon was present in all cases. Dietetic control and restoration of normal mineral balance resulted in a cure in two instances and improvement in the remainder.

*Chemical Studies of Patients with Non-Tropical Sprue* By PAUL STARR, M D , Chicago, Ill

This report consists of (1) a detailed account of the results of diet, thyroid, parathyroid and calcium medication on the metabolic rate, weight, blood calcium and stools of a patient with non-tropical sprue observed from May 1926 to November 1928. (2) the chemical data of calcium balance metabolism experiments on a second patient on low fat, high protein diet, normal high fat diet, and normal low fat diet, covering twenty-four three day periods during five months observations, (3) the effect of gastric digestion on the blood calcium in normal patients with a few observations on patients with sprue, consisting of a tabulation of the change in the blood calcium during a fractional meal in twenty instances. The suggestions in regard to the calcium metabolism of sprue to be drawn from these studies are as follows: (1) Chronic latent tetany is present in non-tropical sprue. (2) it may become acute with extreme depression of the blood calcium, (3) negative calcium balance could not be detected on low fat diets, (4) negative calcium balance was found on a high fat diet during diarrhea, (5) treatment calculated to correct this is beneficial, (6) there is an associated depressed basal metabolic rate, and (7) benefit from raising it by thyroid administration, (8) there is a slight but definite depression of the blood calcium during the gastric digestion of a fractional test-meal, (9) this is not dependent upon acid secretion.

*Hyperparathyroidism* By HAROLD BULGER, M D , HENRY H DIXON, M D (by invitation) and DAVID P BARR, M D , St Louis, Mo

We have studied a patient presenting multiple giant-cell bone tumors, hypotonicity of muscles, bilateral nephrolithiasis, hypercalcemia and abnormal tendency to excrete calcium in the urine and a parathyroid tumor.

The removal of the parathyroid tumor caused severe and almost fatal tetany, hypocalcemia, change in the size of the bone tumors and abnormal tendency to the retention of calcium.

The symptoms of this patient closely resemble many cases which are scattered

*The Veins of the Suprarenal Glands in Patients with Normal and High Blood Pressure A Study of the Musculature and Volume in 100 Cases* By EDGAR V ALLEN, M.D (by invitation) Rochester, Minn

The suprarenal glands removed at autopsy from 50 cases each with normal and high blood pressure were studied with particular reference to the musculature of the veins and the venous vascularization. About 1400 veins were so studied by sectioning the adrenals at areas 1 cm apart, staining with Van Gieson connective tissue stain and projecting at magnifications varying from 50 to 200 times. The areas of the lumens of the veins were then calculated by means of a planimeter and the readings corrected statistically.

The results of this study determine the condition of the musculature of the suprarenal veins in the two clinical conditions and give evidence concerning the amount of vascularization by veins. The relation of the condition of the musculature and clinical hypertension is discussed.

*Repeated Glucose Tolerance Tests* By HENRY J JOHN, M.D., Cleveland, Ohio

The study of borderline cases of diabetes through repeated glucose tolerance tests over a period of years offers interesting observations as to the evolution or the "keeping in check" of diabetes in patients under different living conditions. It also gives us a definite guide as to the degree of improvement in the patient's condition and thus tells us the degree of liberty we can take with his diet. Or again it shows a retrogression, due to carelessness in diet, infections, mental strains or inadequate treatment. Briefly it opens up a study, which after a sufficient length of time ought to throw some light on the fluctuations of the carbohydrate metabolism under varied conditions and what I consider most important—it ought to tell us just what the status of the borderline group of cases is and through that give us a definite idea as to what is the best way of dealing with this group—in other words it will lead us into preventive medicine in the field of diabetes.

*Hyperinsulinism* By FRANK N. ALLAN, M.D. (by invitation), Rochester, Minn

Hyperinsulinism is now recognized as a clinical entity, as a result of the study of a case with a constant tendency to hypoglycemia, previously reported in collaboration with Wilder, Power and Robertson. In this case the cause of the trouble was shown to be overproduction of insulin from a carcinoma originating in the islands of the pancreas. Recently two cases have been under observation, presenting a similar tendency to hypoglycemia, necessitating the ingestion of food or sugar at regular intervals during the day and night to prevent the occurrence of mental confusion, unconsciousness, and convulsions. Hyperinsulinism is suspected, although it cannot be definitely proven. In one case the pancreas appeared normal on surgical exploration, and microscopic examination failed to reveal any abnormality. Removal of a part of the pancreas has diminished but has not completely abolished the tendency to hypoglycemia. The patient no longer requires feeding at night and can remain free of symptoms with only three meals during the day.

characteristic distribution of the fusospirochetes. Neosalvarsan in certain cases if given early has a favorable influence but in most instances of the diffuse types of gangrene the outcome is unfavorable.

*Studies in Syphilis of the Stomach* By HARRY A. SINGER, M.D. (by invitation), Chicago, Ill.

According to current medical literature, there is a marked discrepancy between the incidence of syphilis of the stomach in the morgue as compared with its occurrence in the clinic. The chief reason for this difference is variance in opinion as to what constitutes from the anatomic standpoint gastric lues. Pathologists are inclined to disregard all but those cases in which specific changes are demonstrable. A study of a series of cases from the combined clinical and morphological standpoints indicate that at the time most patients with syphilis of the stomach reach the operating or post-mortem table, the lesions are receding or healing and therefore from the microscopic viewpoint not typical of lues. A gradual transition from the active lesion with its classical luetic picture to the healing and healed lesion can be traced by comparing the histologic slides from a series of resected stomachs.

*The Course of Syphilitic Heart Disease* By FREDRICK A. WILLIUS, M.D., Rochester, Min.

This study was undertaken to attempt to determine the course of and the factors influencing the expectation of life in patients suffering from cardiovascular syphilis.

From a large material it was possible to select only one hundred cases in which a definite history of the onset of syphilis was available, and in which the date and cause of death could be accurately determined.

These cases were divided into three groups: the first group comprising those cases presenting clinical evidence of syphilitic aortitis, there were seventeen cases, all males. The second group presenting clinical evidence of syphilitic aortitis associated with aortic insufficiency, comprised fifty-nine males. The third group comprised cases of thoracic aneurism, of which there were thirty-four, all being males.

The groups are considered separately but in the abstract it seems desirable to present average statistics only.

The average age at which syphilis was contracted was twenty-seven years and the average age at which death occurred from heart disease was fifty-seven years, in other words, thirty years elapsed from the time that the disease was contracted until the patient died of heart disease.

Numerous other points are discussed, viz: the incidence of positive serology of the blood and of the spinal fluid in the various groups, the incidence of associated syphilitic manifestations, the types of cardiac manifestations and the occurrence of associated electrocardiographic phenomena.

*The Veins of the Suprarenal Glands in Patients with Normal and High Blood Pressure A Study of the Musculature and Volume in 100 Cases* By EDGAR V ALLEN, M.D. (by invitation) Rochester, Minn

The suprarenal glands removed at autopsy from 50 cases each with normal and high blood pressure were studied with particular reference to the musculature of the veins and the venous vascularization. About 1400 veins were so studied by sectioning the adrenals at areas 1 cm. apart, staining with Van Gieson connective tissue stain and projecting at magnifications varying from 50 to 200 times. The areas of the lumens of the veins were then calculated by means of a planimeter and the readings corrected statistically.

The results of this study determine the condition of the musculature of the suprarenal veins in the two clinical conditions and give evidence concerning the amount of vascularization by veins. The relation of the condition of the musculature and clinical hypertension is discussed.

*Repeated Glucose Tolerance Tests* By HENRY J. JOHN, M.D., Cleveland, Ohio

The study of borderline cases of diabetes through repeated glucose tolerance tests over a period of years offers interesting observations as to the evolution or the "keeping in check" of diabetes in patients under different living conditions. It also gives us a definite guide as to the degree of improvement in the patient's condition and thus tells us the degree of liberty we can take with his diet. Or again it shows a retrogression, due to carelessness in diet, infections, mental strains or inadequate treatment. Briefly it opens up a study, which after a sufficient length of time ought to throw some light on the fluctuations of the carbohydrate metabolism under varied conditions and what I consider most important—it ought to tell us just what the status of the borderline group of cases is and through that give us a definite idea as to what is the best way of dealing with this group—in other words it will lead us into preventive medicine in the field of diabetes.

*Hyperinsulinism* By FRANK N. ALLAN, M.D. (by invitation), Rochester, Minn

Hyperinsulinism is now recognized as a clinical entity, as a result of the study of a case with a constant tendency to hypoglycemia, previously reported in collaboration with Wilder, Power and Robertson. In this case the cause of the trouble was shown to be overproduction of insulin from a carcinoma originating in the islands of the pancreas. Recently two cases have been under observation, presenting a similar tendency to hypoglycemia, necessitating the ingestion of food or sugar at regular intervals during the day and night to prevent the occurrence of mental confusion, unconsciousness and convulsions. Hyperinsulinism is suspected, although it cannot be definitely proven. In one case the pancreas appeared normal on surgical exploration, and microscopic examination failed to reveal any abnormality. Removal of a part of the pancreas has diminished but has not completely abolished the tendency to hypoglycemia. The patient no longer requires feeding at night and can remain free of symptoms with only three meals during the day.

*Determination of Glucose in Tissues from Normal Hyper- and Hypoglycemia, and Depancreatized Animals* By WILLIAM THALHIMER, M D , and MARGARET C PERRY, A M (by invitation), Milwaukee, Wis

The method used for determining glucose in tissue is new. The accuracy of the method was tested by many duplicate determinations, by the recovery of a known amount of glucose added to tissue at various steps of the procedure, and in other ways. Analyses were made which seemed to give accurate determinations of "true" and "apparent" glucose in tissues. Very little variation in the amount of "true" glucose in tissues was found in the different conditions studied.

*Blood Urobilin* By M A BLANKENHORN, M D , Cleveland, Ohio

Normal urobilin content of human blood has not been described. Urobilin is frequently found in the blood under certain pathological conditions in large amounts, and is a constant finding in small amounts in normal urine. It has been considered a threshold substance. By refinement in technique of the Schlesinger fluorescence test, human blood can be shown to have a fairly constant amount of urobilin. The method is described, and the amount as determined from one hundred and twenty-eight normals is reported.

*The Untoward Effects of Phenylhydrazin in Certain Cases of Polycythemia Vera* H Z GIFFIN, M D , and H M CONNER, M D (by invitation), Rochester, Minn

The histories of three patients are presented, in which the use of phenylhydrazin was followed by fatal outcome. In view of the excellent therapeutic results that have been reported in polycythemia vera following the use phenylhydrazin it seems wise to utter this word of caution with respect to its use. Analysis is made of the characteristics of the cases in which the drug should be used with great caution, if at all.

*Serum Complement Fixation Tests in Blastomycosis and in Monilia Infections*

By O GARCIA, M D (by invitation), St Louis, Mo

Agglutination tests for blastomycosis have already been described by a number of workers. A survey of the literature reveals no successful work on complement fixation in blastomycosis.

In a series of experiments both on blastomycosis and monilia infections, it has been possible to develop antigens which can be used for complement fixation tests.

Positive tests have been secured on clinical cases of blastomycosis and also on monilia infections of the mouth, lungs and gastrointestinal tract.

Animals receiving experimental inoculations with these fungi develop definite complement fixing antibodies.

*The Intradermal Salt Test in Fever* By CHARLES DUDEK, M D (by invitation) and DAVID P BARR, M D , St Louis, Mo

The intradermal salt test has been applied to the study of fever. The disap-

pearance time of the intradermally injected salt is regularly diminished in bacterial fevers and in fevers caused by foreign protein injection. It is unchanged in fevers of equal degree produced by external heat.

*Systemic Histamine-like Reactions in Allergy Due to Cold. A Report of Three Cases.* By BAYARD T. HORTEN, M.D. (by invitation) and GEORGE E. BROWN, M.D., Rochester, Minn.

This report is based on the studies carried out on three subjects, all exhibiting skin disturbances and systemic reactions following exposure to moderate grades of cold. The reactions were so similar in these cases as to constitute a most striking clinical syndrome, which, to our knowledge, has never been reported.

Three cases are reported exhibiting local and general symptoms of cold allergy. The local effects on the skin were pallor during the exposure period followed by redness, swelling, increased local heat of the hands after exposure to cold. Following a latency period of three to four minutes, a characteristic systemic reaction appeared, which was quite characteristic of that produced by histamine. There was a fall in blood pressure, a sharp rise in pulse rate, a tendency to syncope, with transitory recovery in fifteen to thirty minutes. Complete recovery from the local reaction occurred in twelve to twenty-four hours. The experimental work carried out gave evidence of the chemical nature of these reactions, and strongly suggested the release of a histamine-like substance in the skin following exposure to cold which, when carried in the general circulation, produced a reaction characteristic of histamine. These clinical observations are further confirmation of the work of Lewis and associates on the presence of histamine or allied substances in the human skin.

*The Relation of the Weather to the Pain of Arthritis.* By E. B. RENTSCHLER, M.D. (by invitation), FRANCES VANZANT, M.D. (by invitation) and L. G. ROWNTREE, M.D., Rochester, Minn.

It has been found that in an arthritic medical service, the pain of infectious arthritis varies markedly with the changes in the weather. The condition of each patient on the service has been reported every day for a year. On completing rounds the doctors on the arthritic service have made a summary of the statements of the patients and recorded the results. At the same time records have been kept in the laboratory of barometric pressure and temperature inside and outside, humidity, sunshine, the presence or absence of storms and the electrical state.

A remarkable correlation has been found between the pain of arthritis and the presence of a storm. In fact, there were only two or three storms in the entire year not reflected in the pain of the patients with arthritis. There is also a remarkable correlation between the changes in barometric pressure and the pain of arthritis. A positive correlation in more than 70 per cent and some sort of correlation in more than 90 per cent of cases has been observed. The amount of

sunshine also exercises a marked influence. Certain patients claim a capacity to predict storms by their pain and have been checked, and one has been found to be correct over 95 per cent of the time. In patients who believed that the weather in no way influenced their pain almost as striking a correlation was found at times. This study brings up many considerations which have largely been overlooked by the physician.

*Urinary Proteins* By WILLIAM A. THOMAS, M.D. (by invitation), Chicago, Ill.

In the production of artificial uremia in dogs, there is obtained a protein in the urine which does not originate in the blood. This protein has been identified by appropriate precipitation tests as coming from viscera and muscle and is definite in its immunological reactions.

Later, this disappears and is replaced by serum protein in large quantities. Demonstration of this protein from several organs has been successful.

Later the process so far breaks down this protein that it is not recognizable as such, but combines with serum protein and is excreted in this combination.

The mechanism of excretion of protein through the kidney damaged or undamaged is discussed and it is believed that the kidney is not permeable to protein which belongs in the circulation of that species but is excreted only when altered by chemical, osmotic or other factors to such an extent that it is no longer the normal serum protein of that organism and that the liver protein combines with serum protein as a mechanism of detoxication and excretion.

*Symptomatic Relapses During Liver-Induced Remissions in Pernicious Anemia*

By CYRUS C. STRUGIS, M.D., and RAPHAEL ISAACS, M.D. (by invitation), Ann Arbor, Mich.

Many patients with pernicious anemia whose blood has returned to normal while taking liver or an adequate liver fraction, experience, during the months following the development of the remission, certain symptoms which are similar to those of a beginning relapse. These include nausea, loss of appetite, weakness, nervousness, diarrhea or constipation, increase in "numbness" of hands and feet, sore tongue and ease of fatigue. If the liver or liver extract is continued until these symptoms pass away or decrease, a true "hematological" relapse is prevented. Some patients discontinue the liver at this time and the anemia reappears. It is evident that the active liver component acts by supplying a substance, the power to elaborate which has been lost, and is never regained. The treatment is symptomatic and does not influence the fundamental disease process. The periodicity of the relapses and remissions remains a characteristic, even though the blood (and bone marrow) have been restored to normal function.

## COMPOSITION OF THE DIETS EMPLOYED

*Diet A*

| Substance           | Weight | Protein | Fat   | Carbo-<br>hydrate | Calories | Ca    | P     |
|---------------------|--------|---------|-------|-------------------|----------|-------|-------|
|                     | grams  | grams   | grams | grams             |          | grams | grams |
| Egg                 | 100    | 13.4    | 10.5  |                   | 148.0    | 0.067 | 0.180 |
| Bread               | 30     | 2.8     | 0.4   | 15.8              | 77.7     | 0.008 | 0.028 |
| Butter              | 10     | 0.1     | 8.5   |                   | 76.9     | 0.002 | 0.002 |
| Lettuce             | 30     | 0.4     | 0.1   | 0.9               | 5.7      | 0.013 | 0.013 |
| Fresh tomato        | 50     | 0.5     | 0.2   | 2.0               | 11.5     | 0.006 | 0.013 |
| Sugar               | 10     |         |       | 10.0              | 40.0     |       |       |
| Egg                 | 20     | 2.7     | 2.1   |                   | 29.6     | 0.013 | 0.036 |
| Cream (18 per cent) | 100    | 2.5     | 18.5  | 4.5               | 195.0    | 0.086 | 0.067 |
| Total               |        | 22.4    | 40.3  | 33.2              | 584.4    | 0.195 | 0.339 |

## ERRATUM

When binding this volume insert the attached tables in Volume VII, Number 1 after page 96

*Diet C*

| Substance           | Weight | Protein | Fat   | Carbo-<br>hydrate | Calories | Ca    | P     |
|---------------------|--------|---------|-------|-------------------|----------|-------|-------|
|                     | grams  | grams   | grams | grams             |          | grams | grams |
| Egg                 | 50     | 6.7     | 5.3   |                   | 74.0     | 0.034 | 0.090 |
| Egg yolk            | 100    | 15.7    | 33.3  |                   | 363.0    | 0.137 | 0.567 |
| Cream (18 per cent) | 50     | 1.3     | 9.3   | 2.3               | 77.5     | 0.073 | 0.017 |
| Sugar               | 10     |         |       | 10.0              | 40.0     |       |       |
| Apple               | 100    | 0.4     | 0.5   | 14.2              | 63.0     | 0.007 | 0.012 |
| Egg white           | 30     | 3.7     | 0.1   |                   | 15.3     | 0.005 | 0.004 |
| Shredded Wheat      | 35     | 4.2     | 0.6   | 26.3              | 127.8    | 0.014 | 0.103 |
| Milk                | 200    | 6.6     | 8.0   | 10.0              | 138.0    | 0.240 | 0.186 |
| Sugar               | 10     |         |       | 10.0              | 80.0     |       |       |
| Total               |        | 38.6    | 57.1  | 72.8              | 958.6    | 0.480 | 0.939 |

*Diet D*

| Substance           | Weight | Protein | Fat   | Carbo-<br>hydrate | Calories | Ca     | P      |
|---------------------|--------|---------|-------|-------------------|----------|--------|--------|
|                     | grams  | grams   | grams | grams             |          | grams  | grams  |
| Egg (whole)         | 170    | 25.8    | 17.9  |                   | 251.6    | 0.1139 | 0.3060 |
| Egg (white)         | 30     | 3.7     | 0.1   |                   | 15.3     | 0.0045 | 0.0050 |
| Egg (yolk)          | 100    | 15.7    | 33.3  |                   | 363.0    | 0.1370 | 0.5240 |
| Cream (18 per cent) | 110    | 2.8     | 20.4  | 5.0               | 214.5    | 0.0946 | 0.0737 |
| Sugar               | 30     |         |       | 30.0              | 120.0    |        |        |
| Apple               | 100    | 0.4     | 0.5   | 14.2              | 63.0     | 0.0070 | 0.0120 |
| Milk                | 700    | 23.1    | 28.0  | 35.0              | 483.0    | 0.8400 | 0.6510 |
| Shredded Wheat      | 35     | 4.2     | 0.6   | 26.3              | 127.8    | 0.0144 | 0.1134 |
| Butter              | 50     | 0.5     | 42.5  |                   | 384.5    | 0.0045 | 0.0085 |
| Tenderloin          | 100    | 16.2    | 24.4  |                   | 284.0    | 0.0093 | 0.1725 |
| Lettuce             | 40     | 0.5     | 0.1   | 1.2               | 7.6      | 0.0172 | 0.0168 |
| Asparagus           | 100    | 1.8     | 0.2   | 3.3               | 22.0     | 0.0250 | 0.0390 |
| Bread               | 80     | 7.4     | 1.0   | 42.2              | 207.2    | 0.0216 | 0.0744 |
| Celery              | 40     | 0.4     |       | 1.3               | 7.6      | 0.0312 | 0.0148 |
| Cauliflower         | 100    | 1.8     | 0.5   | 4.7               | 31.0     | 0.1230 | 0.0610 |
| Brussels            | 100    | 1.3     | 0.6   | 22.0              | 99.0     | 0.0090 | 0.0310 |
| Total               |        | 102.6   | 170.1 | 185.2             | 2,681.1  | 1.45   | 2.1023 |
| Analyzed            |        |         |       |                   |          | 0.994  | 1.756  |

*Diet L*

| Substance           | Weight | Protein | Fat   | Carbo-<br>hydrate | Calories | Ca     | P      |
|---------------------|--------|---------|-------|-------------------|----------|--------|--------|
|                     | grams  | grams   | grams | grams             |          | grams  | grams  |
| Apple               | 330    | 1.3     | 1.7   | 46.9              | 207.9    | 0.0231 | 0.0396 |
| Sugar               | 39     |         |       | 39.0              | 156.0    |        |        |
| Egg white           | 120    | 14.8    | 0.2   |                   | 61.2     | 0.0180 | 0.0168 |
| Cream (40 per cent) | 195    | 4.3     | 78.0  | 5.9               | 743.0    | 0.1677 | 0.1307 |
| Bread               | 195    | 18.7    | 2.7   | 99.6              | 498.3    | 0.0527 | 0.1814 |
| Butter              | 75     | 0.8     | 63.8  |                   | 576.8    | 0.0113 | 0.0128 |
| Total               |        | 39.9    | 146.4 | 191.4             | 2,243.2  | 0.273  | 0.381  |
| Analyzed            |        |         |       |                   |          | 0.218  | 0.345  |

## STUDIES OF SERUM ELECTROLYTES

### IV THE CHLORIDE AND NITROGEN BALANCES, AND WEIGHT CHANGES IN PNEUMONIA<sup>1</sup>

By F WILLIAM SUNDERMAN<sup>2</sup>

*(From the John Herr Musser Department of Research Medicine, University of Pennsylvania and the Medical Wards<sup>3</sup> of the Pennsylvania Hospital Philadelphia)*

(Received for publication October 30, 1928)

In an earlier study (1) data were presented on the changes in the blood serum during the course of lobar pneumonia. It was found that the concentration of total base, chloride, and protein in the serum was usually decreased during the active infection. Following the crisis these concentrations returned to their normal values, the chloride less rapidly than the total base. The low excretion of chloride in the urine during the precritical period has long been known and the inference has been drawn that there is a correlation between the urinary excretion and the serum concentration. No satisfactory explanation of the mechanism of the change in composition of the serum or of the decreased urinary excretion of chloride has been established, but from studies in the literature it has often been inferred that during the precritical period the body tissues retain chloride which is subsequently released during the epicritical period. In an attempt to obtain data on factors concerned in these changes, studies of the intake and output were undertaken in patients suffering with lobar pneumonia.

To review completely the very extensive literature upon the chloride concentration of the serum and urine in pneumonia seems unnecessary since this has been done by many authors, notably by Hutchison (6), Von Moraczewski (8),

---

<sup>1</sup> Aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

<sup>2</sup> Robert M. Girvin Fellow in Research Medicine.

<sup>3</sup> Service of Dr. George W. Norris.

Garratt (7), and Peabody (9) The conclusion of the earlier workers that chloride was retained in the body during the febrile period arose chiefly from the observation that the addition of chloride to the diet during this period was not accompanied by a corresponding increase of chloride excretion These observations were later supplemented by analyses of tissues and studies of chloride balance Von Moraczewski (8) reported 18 cases of pneumonia in which daily amounts of chloride, nitrogen, phosphorus, and calcium in the food, urine, and feces were determined Only three times in his series, twice when the food intake was very small and once when calcium phosphate was administered, did he find the daily excretion of chloride in the urine and feces in excess of the daily intake of chloride He observed that although the excretion of chloride in pneumonia parallels in general the intake of chloride, nevertheless, an excessive ingestion of salt was not accompanied by the normally prompt excretion

Hutchison (6) reported 4 cases that excreted less chloride in the urine during the febrile period than was taken with the food, but these patients received from 10 to 75 grams of chloride per day Hutchison found no evidence from tissue analyses that chloride tended to accumulate in any particular organ during pneumonia, but rather that all of the organs seemed to contain somewhat more chloride per unit of weight than normally

In order to determine how much chloride could be retained in the pneumonic exudate, Peabody (9) analyzed the lungs in two cases of pneumonia in which one lung was normal and the other hepatized He found that a retention of about two grams of sodium chloride could be credited to the pneumonic lung The skin of pneumonia patients showed no significant increase in its salt content Von Hosslin (10) averaged the percentile concentration of chloride in normal and pneumonic lungs from his own data and from data of Jarish (11), Meillère (12), Hutchison (6), and Terray (13) and found that the mean sodium chloride concentration in pneumonic lungs in 14 observations was 0.343 per cent of fresh weight and in 10 estimations in normal lungs, 0.331 per cent Taking the weight of a normal lung at 250 grams and a hepatized lung at 1250 grams, there would be an increase of about 3.5 grams of sodium chloride in the latter

Sandelowsky (2) gave from 3 to 36 grams of sodium chloride in 3 days to patients convalescing from pneumonia He used himself as a control and observed in the pneumonia patients a greater delay in the excretion of the sodium chloride

Prigge (14) upon injecting from 100 to 150 cc. of a 25 per cent solution of sodium chloride intravenously into patients during the precritical period of lobar pneumonia also observed a lagging in the excretion of the chloride, although the blood concentration fell the day following injection and thus behaved as in normal individuals

Holten (15) fed pneumonia patients during the precritical period a diet which contained between 6 and 7 grams of sodium chloride daily and found that less than the corresponding amount of chloride was excreted in the urine until about the time of crisis

Leyden (5) in 1869 was perhaps the first to study weight changes in pneumonia. He observed in his patients an increased loss of weight through the skin and lungs during the febrile period and a rapid loss following the crisis. Sandelowsky (2) studied the body weights in 11 cases of pneumonia. In 7 cases the body weight either increased slightly during the precritical period or remained constant, whereas, after the crisis the weight fell more or less rapidly, in 4 there was a continued decrease in weight during the febrile and afebrile periods.

Garnier and Sabareanu (3) observed that pneumonia patients do not always lose weight in the early stages of the disease but that even with a small food intake they sometimes gain weight. The gain in weight in their cases did not generally exceed 500 grams per day but was in one instance as high as 2000 grams in three days. With fall in temperature the weight dropped. In children suffering with pneumonia the gain in weight before the crisis would appear to occur more frequently than in adults. Lussky and Friedstein (4) observed in their series of children that the weight remained constant or was increased before crisis, but was decreased with the disappearance of fever. Only one out of their 28 cases occurring in children failed to show loss of weight following the crisis.

#### MATERIAL AND METHODS

These studies were carried out on seven patients with lobar pneumonia. In five patients study was commenced between the fifth and eighth days of the disease. Two patients, selected because they were admitted on the third day of the disease, were treated with large doses of sodium chloride by mouth. The period of investigation for each patient was from 2 to 11 days and except in one fatal case extended 1 to 8 days following the crisis. The patients selected were young adult males who had all been healthy before the onset of pneumonia. In addition two normal individuals were followed by similar methods of study for 48 hours on a diet comparable with that received by the patients. The normal individuals were subjected each day to a cabinet light bath designed to promote a loss of water through the skin which would approach in magnitude that occurring in the patients.

On admission the patients were placed in a special metabolic room and were under constant observation during the period of study. One patient or occasionally two being studied at a time. Each morning at 7 o'clock the patient was weighed. A silk scale of the type proposed by Benedict and Root (16), which weighs accurately to 10 grams, was used and was fitted with an especially constructed thin stretcher. With the exception of the first 2 days of study in Case B3, no patient received any food for at least 8 hours before weighing time, and none received water or food between 5.30 and 7 o'clock in the morning. The patient was encouraged to defecate and void during the half hour before weighing. If unable to void, he was catheterized.

The daily intake consisted of weighed amounts of the following ingredients: milk, grape juice, tea, canned tomato and pea purees, triscuit, sugar, salt and water. The diet corresponded to the type given to the hospital patients suffering

from lobar pneumonia but was designed to contain articles which have an approximately uniform chemical analysis. Analyses of the commercial foods were furnished to us by the respective manufacturers, these were supplemented by our own analyses and by values taken from the Food Bulletin of the Connecticut Agricultural Experiment Station (17). The chloride content of the foods was analyzed.

In one patient (B3) a high caloric diet consisting of cream, milk, eggs, and malted milk was given for 2 days.

Enemas that were given contained small amounts of soap.

The collected measured output included urine, feces, sputum, and blood removed for analysis. These were weighed to a gram. Toluol was used to preserve the urine. The excreta were kept refrigerated. An attempt to collect the sweat was unsatisfactory.

The dry weights of excreta and the nitrogen contents of the urine and feces were determined. The total amount of nitrogen excreted per day in the sputum was found in two analyses to be negligibly small. The chlorides were analyzed according to Van Slyke's method (18) in serum and food and Volhard's in urine. The Kjeldahl method was used for nitrogen determination.

## RESULTS

In table 1 are given data on the food intake, nitrogen and chloride balance and changes of weight. The day of crisis is designated as the zero day and days before and after as - and + respectively. An estimate of the caloric requirement has been made from normal basal standards for weight and age with an addition for fever, estimated by the average percentile increase of metabolism per degree increase of temperature from Du Bois' data (19) and an added 10 per cent during the precritical period. The caloric value of the diets was consistently low and the nitrogen balance markedly negative. An attempt to increase the intake of food in patient B3 led to serious tympanites and for this series of studies such attempts were not repeated. Patients B6 and B7, who were given large doses of sodium chloride, took more food spontaneously during the precritical period without deleterious symptoms.

### *Weight changes*

Loss of weight was usually present in both precritical and post-critical periods as would be expected with such an inadequate diet. In table 2 is shown the relative daily loss of weight in grams per kilogram of initial weight and the negative nitrogen balance per kilogram

of initial weight for the patients studied and, for comparison, similar data from a series of fasting experiments from Benedict's (20) collection of studies. The weight curve during the postcritical period of B5 was possibly modified by the development of a pleural effusion. B6 and B7 received large amounts of NaCl which in B7 were poorly excreted and led eventually to slight visible edema. Patients B3 and B4 exhibited increased loss of weight following the crisis associated with unchanged or with less negative nitrogen balance and less caloric deficit. A similar increased loss of weight following the crisis was noted by Leyden (5) and interpreted by him to indicate precritical storage of water and postcritical release. The behavior of the weight curves resembles that observed in fasting studies. The fluctuations in the rate of loss of weight from day to day can be approximately duplicated in fasting studies. Partial inanition is evidently an important factor in inducing the observed changes in weight with apparently additional factors diminishing the rate of loss of weight before the crisis, and increasing it after the crisis.

That nitrogen equilibrium is not maintained in pneumonia has been demonstrated by many workers, including von Moraczewski (8), Cook (21), and Leyden and Klemperer (22). The negative nitrogen balance in the pneumonia patients was higher than in fasting subjects doubtless because of the greater metabolism in the former. The loss of weight per milligram of negative nitrogen balance was less in the precritical periods in all the patients and throughout the study of B5 and in the two subjects receiving salt than in the fasting subjects. This might be considered indirect evidence of relative retention of water during the precritical period. In the postcritical periods of B3 and B4 the loss of weight was closely comparable with that observed in the fasting subjects.

#### *Excretion through skin and lungs*

In table 1 are given the data on the urinary loss of water and the loss of weight through skin and lungs. The loss of water through skin and lungs is equal to the total loss of weight through skin and lungs minus the difference between weight of  $\text{CO}_2$  given off and of  $\text{O}_2$  absorbed through the lungs. This difference of weight is about  $75 \pm 75$  grams per day. It is evident from these data that from 25

TABLE I  
Data on cases

| Case number | Day from crisis | Temperature° | Initial weight and daily change | Urinary water | Weight loss skin and lungs | Intake  |              |     |          | Estimated caloric deficit | Nitrogen output | Nitrogen balance | Chloride intake | Chloride output |       |        |                                  |       | Chloride balance | Expected chloride balance<br>= 50 per cent | Relative chloride balance | Amount of sputum |          |    |     |
|-------------|-----------------|--------------|---------------------------------|---------------|----------------------------|---------|--------------|-----|----------|---------------------------|-----------------|------------------|-----------------|-----------------|-------|--------|----------------------------------|-------|------------------|--|---------------------------|------------------|----------|----|-----|
|             |                 |              |                                 |               |                            | Protein | Carbohydrate | Fat | Calories |                           |                 |                  |                 | Urine           | Feces | Sputum | In blood removed<br>for analysis | Total |                  |  |                           |                  |          |    |     |
| B1          | -1              | 103          | 50, 166                         | 359           | 1,002                      | 3,040   | 25           | 77  | 38       | 755                       | 1,400           | 17               | -13             | 17              | 0     | 2      | 5                                | 5     | 1                | 3  | 0                         | 20 ± 9           | -3 ± 9   | ?  | 88  |
|             | 0               | 104          | -                               | 396           | 1,021                      | 2,744   | 26           | 102 | 31       | 799                       | 1,300           | 20               | -16             | 17              | 0     | 0      |                                  | 11    | 6                | 4  | 6                         | 25 ± 9           | -8 ± 9   | ?  | 152 |
|             | +1              | 98           | -                               | 1,412         | 999                        | 2,711   | 30           | 87  | 35       | 786                       | 800             | 22               | -17             | 21              | 0     | 7      | 1                                | 3     | 5                | 8  |                           | 17 ± 9           | +1 ± 9   | +  | 86  |
|             | -3              | 105          | 48, 138                         | -             | -                          | -       | -            | -   | -        | -                         | -               | -                | -               | 36              | 9     |        | 2                                | 8     | 4                | 2  |                           | 53 ± 9           | -17 ± 10 | -  | 35  |
| B3          | -3              | 104          | 86, 138                         | 186           | 1,473                      | 2,436   | 86           | 215 | 153      | 2,733                     | 900             | 26               | -12             | 137             | 18    | 7      |                                  | 8     | 1                | 4  | 0                         | 40 ± 9           | +97 ± 12 | +  | 108 |
|             | -2              | 104          | -                               | 640           | 1,494                      | 2,174   | 46           | 118 | 74       | 1,412                     | 2,200           | 27               | -20             | 89              | 10    | 8      | 2                                | 4     | 3                | 7  | 5                         | 31 ± 9           | +58 ± 11 | +  | 57  |
|             | -1              | 103          | -                               | 1,260         | 1,616                      | 2,197   | 0            | 8   | 0        | 333                       | 200             | 26               | -26             | 0               | 4     | 1      |                                  | 3     | 8                |  | 17 ± 9                    | -17 ± 9          | ?        | 50 |     |
|             | 0               | 105          | -                               | 1,106         | 1,327                      | 4,276   | 12           | 52  | 14       | 391                       | 3,100           | 23               | -21             | 7               | 2     | 9      |                                  | 2     | 8                |  | 15 ± 9                    | -8 ± 9           | +        | 43 |     |
|             | +1              | 100          | -                               | 610           | 1,813                      | 2,584   | 41           | 141 | 47       | 1,160                     | 1,600           | 36               | -29             | 27              | 3     | 3      | 0                                | 3     | 1                | 6  | 3                         | 18 ± 9           | +9 ± 10  | +  | 12  |
|             | +2              | 100          | -                               | 623           | 2,067                      | 2,080   | 52           | 110 | 63       | 1,227                     | 1,600           | 27               | -19             | 39              | 11    | 4      |                                  | 2     | 6                |  | 23 ± 9                    | +16 ± 10         | +        | 31 |     |
|             | +3              | 100          | -                               | 1,324         | 2,964                      | 2,382   | 48           | 100 | 57       | 1,117                     | 1,700           | 31               | -23             | 111             | 57    | 3      | 1                                | 7     | 1                | 8  | 3                         | 74 ± 9           | +37 ± 11 | +  | 20  |
|             | +4              | 98           | -                               | 1,141         | 2,715                      | 2,274   | 64           | 143 | 74       | 1,500                     | 800             | 26               | -16             | 98              | 76    | 3      | 1                                | 4     | 3                | 4  | 90 ± 9                    | +7 ± 11          | +        | 40 |     |
| +5          | 98              | -            | 554                             | 2,306         | 1,829                      | 33      | 132          | 36  | 1,002    | 1,300                     | 23              | -18              | 125             | 155             | 0     |        | 0                                | 5     |                  | 165 ± 9                                    | -40 ± 12                  | ?                | 6        |    |     |
| +6          | 98              | -            | 1,559                           | 2,259         | 1,997                      | 35      | 148          | 35  | 1,060    | 1,200                     | 20              | -14              | 74              | 164             | 0     | 1      | 5                                |       |                  | 3  | 178 ± 9                   | -104 ± 10        | -        | 0  |     |
| +7          | 98              | -            | 639                             | 1,919         | 1,690                      | 11      | 100          | 9   | 555      | 1,700                     | 17              | -15              | 86              | 134             | 0     |        |                                  |       |                  |  | 143 ± 9                   | -58 ± 11         | -        | 0  |     |

|    |    |     |        |        |     |           |       |     |     |       |     |       |     |  |  |      |        |          |     |   |     |
|----|----|-----|--------|--------|-----|-----------|-------|-----|-----|-------|-----|-------|-----|--|--|------|--------|----------|-----|---|-----|
| B4 | -2 | 104 | 60,934 | 87     | 37  | 800,1900  | 32    | -27 | 22  | 39.5  |     |       |     |  |  | 50   | 54 ±9  | -32 ±9   | +5  | - | 0   |
|    | -1 | 103 | +      | 104    | 42  | 939,1700  | 32    | -26 | 25  | 31.6  | 0.6 |       |     |  |  | 1.8  | 45 ±9  | -20 ±10  | +5  | - | 60  |
|    | 0  | 102 | -      | 90     | 40  | 861,1600  | 32    | -27 | 25  | 37.6  |     |       |     |  |  | 1.0  | 51 ±9  | -26 ±10  | -71 | + | 26  |
|    | +1 | 99  | -      | 93     | 34  | 798,1400  | 26    | -22 | 20  | 21.0  | 3.6 |       |     |  |  | 2.8  | 36 ±9  | -17 ±9   | -68 | + | 0   |
|    | +2 | 99  | -      | 75     | 33  | 698,1500  | 22    | -18 | 20  | 17.7  |     |       |     |  |  |      | 27 ±9  | -7 ±9    | -35 | + | 8   |
|    | +3 | 99  | +      | 107    | 41  | 936,1300  | 24    | -19 | 24  | 16.7  | 1.9 |       |     |  |  | 0.3  | 30 ±9  | -6 ±9    | +5  | ? | 7   |
|    | +4 | 98  | -      | 124    | 41  | 014,800   | 17    | -11 | 24  | 29.0  |     |       |     |  |  |      | 38 ±9  | -14 ±9   | -38 | ? | 3   |
|    | +5 | 98  | -      | 33     | 35  | 943,900   | 18    | -13 | 56  | 21.7  |     |       |     |  |  | 3.3  | 34 ±9  | +22 ±10  | -20 | + | 5   |
|    | +6 | 98  | +      | 212    | 48  | 1,508,300 | 17    | -14 | 143 | 48.9  |     |       |     |  |  | 0.7  | 59 ±9  | +84 ±12  | +10 | + | 11  |
|    | +7 | 98  | +      | 188    | 172 | 2,707     | 21    | -5  | 152 | 164.0 | 0.9 |       |     |  |  | 2.2  | 176 ±9 | -24 ±12  | +10 | - | 0   |
|    | +8 | 98  | -      | 155    | 113 | 1,924     | 23    | -13 | 121 | 171.0 | 2.1 |       |     |  |  |      | 182 ±9 | -61 ±11  | -28 | - | 0   |
| B5 | -3 | 103 | -      | 26 528 | 92  | 42        | 890   | 600 | 13  | -7    | 26  | 22.9  | 2.1 |  |  | 0.1  | 2.0    | -11 ±10  | -7  | ? | 24  |
|    | -2 | 104 | -      | 30     | 83  | 36        | 773   | 800 | 14  | -9    | 22  | 30.2  | 0.4 |  |  | 2.9  |        | -21 ±9   | -10 | ? | 36  |
|    | -1 | 103 | -      | 27     | 94  | 32        | 782   | 700 | 11  | -7    | 19  | 13.6  |     |  |  | 4.6  | 3.1    | -11 ±9   | -2  | ? | 47  |
|    | 0  | 103 | -      | 27     | 103 | 32        | 840   | 700 | 14  | -10   | 19  | 13.2  | 3.0 |  |  | 3.8  |        | -11 ±9   | -26 | ? | 45  |
|    | +1 | 100 | -      | 24     | 92  | 26        | 707   | 400 | 9   | -5    | 43  | 4.1   | 0.9 |  |  | 2.4  | 3.3    | +23 ±10  | -14 | + | 28  |
|    | +2 | 100 | +      | 46     | 187 | 45        | 1,361 |     | 8   | -1    | 94  | 4.9   |     |  |  | 1.4  |        | +78 ±11  | +26 | + | 19  |
|    | +3 | 100 | +      | 56     | 111 | 98        | 1,557 |     | 11  | -2    | 132 | 9.4   | 3.4 |  |  | 0.1  |        | +110 ±12 | +14 | + | 10  |
| B6 | -5 | 102 | -      | 50,625 | 116 | 48        | 1,063 | 900 | 18  | -12   | 137 | 17.3  |     |  |  | 4.6  | 3.5    | +103 ±12 | -16 | + | 48  |
|    | -1 | 101 | +      | 53     | 130 | 64        | 1,303 | 500 | 21  | -16   | 211 | 37.0  | 1.5 |  |  | 14.2 |        | +149 ±13 | +26 | + | 97  |
|    | -3 | 101 | +      | 55     | 137 | 63        | 1,340 | 500 | 15  | -6    | 296 | 112.0 | 0.6 |  |  | 10.4 | 5.5    | +158 ±15 | +3  | + | 86  |
|    | -2 | 101 | -      | 46     | 158 | 58        | 1,369 | 400 | 16  | -13   | 291 | 231.0 | 1.3 |  |  | 9.5  | 4.2    | +36 ±15  | -19 | + | 64  |
|    | -1 | 101 | -      | 49     | 148 | 59        | 1,340 | 500 | 14  | -6    | 527 | 346.0 | 1.0 |  |  | 14.2 |        | +157 ±20 | -2  | + | 119 |
|    | 0  | 101 | -      | 45     | 157 | 51        | 1,299 | 500 | 12  | -5    | 344 | 316.0 |     |  |  | 6.3  | 4.3    | +8 ±16   | -24 | + | 60  |
|    | +1 | 100 | -      | 50     | 138 | 57        | 1,270 | 400 | 13  | -5    | 332 | 288.0 | 0.3 |  |  | 6.5  |        | +28 ±16  | -12 | + | 54  |
|    | +2 | 100 | -      | 51     | 152 | 60        | 1,370 | 300 | 13  | -5    | 315 | 292.0 | 0.3 |  |  | 6.5  | 4.3    | +3 ±15   | -21 | ? | 49  |
|    | +3 | 100 | -      | 48     | 127 | 51        | 1,187 | 500 | 14  | -6    | 224 | 295.0 | 0.6 |  |  | 7.7  |        | -88 ±13  | -31 | - | 52  |
|    | +4 | 100 | -      | 45     | 105 | 53        | 1,090 | 600 | 12  | -5    | 351 | 192.0 | 0.2 |  |  | 3.4  |        | +146 ±16 | -26 | + | 24  |

TABLE 1—Continued

| Case number           | Day from crisis | Temperature* | Initial weight and daily change | Urinary water | Weight loss skin and lungs | Intake  |              |     |          | Estimated caloric deficit | Nitrogen output | Nitrogen balance | Chloride intake | Chloride output |       |        |                  |       | Chloride balance | Expected chloride balance<br>±50 per cent | Relative chloride balance | Amount of sputum |      |     |     |     |
|-----------------------|-----------------|--------------|---------------------------------|---------------|----------------------------|---------|--------------|-----|----------|---------------------------|-----------------|------------------|-----------------|-----------------|-------|--------|------------------|-------|------------------|---|---------------------------|------------------|------|-----|-----|-----|
|                       |                 |              |                                 |               |                            | Protein | Carbohydrate | Fat | Calories |                           |                 |                  |                 | Urine           | Feces | Sputum | In blood removed | Total |                  |   |                           |                  |      |     |     |     |
| B7                    | -6              | 104          | 59,278                          | 0             | 1,107                      | 1,703   | 39           | 113 | 46       | 1,030                     | 1,400           | 10               | -4              | 96              | 2     | 0      | 15               | 1     | 3                | 8   | 30                        | ±9               | +66  | ±11 | 0   | 119 |
|                       | -5              | 102          | +                               | 711,090       | 2,113                      | 44      | 97           | 53  | 53       | 1,050                     | 1,100           | 12               | -5              | 203             | 2     | 9      | 11               | 2     |                  |   | 29                        | ±9               | +174 | ±13 | 0   | 90  |
|                       | -4              | 100          | +                               | 914           | 2,115                      | 1,278   | 52           | 108 | 63       | 1,207                     | 900             | 25               | -17             | 296             | 24    | 8      | 10               | 9     | 4                | 1   | 49                        | ±9               | +247 | ±15 | +32 | 80  |
|                       | -3              | 100          | +                               | 129           | 1,932                      | 1,658   | 46           | 109 | 57       | 1,137                     | 1,000           | 19               | -12             | 291             | 71    | 5      | 12               | 7     | 4                | 1   | 104                       | ±9               | +187 | ±15 | +5  | 113 |
|                       | -2              | 100          | +                               | 1,211         | 1,952                      | 795     | 42           | 122 | 51       | 1,137                     | 1,000           | 12               | -5              | 530             | 116   | 6      | 13               | 1     |                  |   | 139                       | ±9               | +391 | ±20 | +42 | 100 |
| Normal subjects<br>B8 | -1              | 100          | +                               | 1,602         | 1,293                      | 989     | 42           | 122 | 51       | 1,137                     | 1,000           | 18               | -11             | 344             | 83    | 4      | 13               | 8     | 4                | 5   | 113                       | ±9               | +232 | ±16 | +56 | 106 |
|                       | 0               | 99           | +                               | 1,715         | 983                        | 872     | 47           | 103 | 57       | 1,117                     | 900             | 10               | -2              | 239             | 47    | 0      | 9                | 4     |                  |   | 67                        | ±9               | +172 | ±11 | +60 | 72  |
|                       | +1              | 99           | +                               | 340           | 1,004                      | 1,018   | 29           | 87  | 35       | 790                       | 1,200           | 9                | -4              | 41              | 38    | 4      | 12               | 5     | 4                |   | 65                        | ±9               | -24  | ±10 | +12 | 83  |
|                       | +2              | 98           | -                               | 354           | 1,022                      | 802     | 20           | 71  | 23       | 595                       | 1,300           | 8                | -5              | 34              | 39    | 5      | 10               | 2     |                  | 60  | ±9                        | -26              | ±10  | -12 | 78  |     |
|                       | +3              | 98           | -                               | 711           | 1,097                      | 868     | 36           | 92  | 43       | 910                       | 1,000           | 9                | -3              | 69              | 40    | 9      | 10               | 6     |                  | 61  | ±9                        | +8               | ±10  | -2  | 76  |     |
| B9                    | 1               |              | 68,046                          |               |                            |         |              |     |          |                           |                 |                  |                 |                 |       |        |                  |       |                  |   |                           |                  |      |     |     |     |
|                       | 2               |              | -                               | 865           | 1,336                      | 2,502   | 37           | 81  | 40       | 830                       | 1,500           | 13               | -10             | 34              |       |        |                  |       |                  |   |                           |                  |      |     |     |     |
|                       |                 |              | -                               | 482           | 1,059                      | 2,092   | 39           | 85  | 41       | 850                       | 1,400           | 13               | -7              | 34              |       |        |                  |       |                  |   |                           |                  |      |     |     |     |
|                       |                 |              | 74,184                          |               |                            |         |              |     |          |                           |                 |                  |                 |                 |       |        |                  |       |                  |   |                           |                  |      |     |     |     |
|                       | 1               |              | -                               | 1,411         | 2,703                      | 2,079   | 36           | 70  | 38       | 765                       | 1,700           | 11               | -5              | 17              |       |        |                  |       |                  |   |                           |                  |      |     |     |     |
|                       | 2               |              | -                               | 843           | 1,676                      | 2,034   | 36           | 75  | 40       | 800                       | 1,700           | 13               | -7              | 34              |       |        |                  |       |                  |   |                           |                  |      |     |     |     |

\* Average of maximum and minimum temperatures for the day

TABLE 2

*Weight and nitrogen balance in pneumonia and in fasting subjects (from literature)*

| Subject               | Period days  | Loss of weight, grams per kilogram per day |         |         | Negative N balance mgm. per kilogram per day |         |         | Weight loss<br>N balance | Caloric deficit<br>Caloric need |
|-----------------------|--------------|--|---------|---------|--|---------|---------|--------------------------|---------------------------------|
|                       |              | Mean for period                            | Maximum | Minimum | Mean for period                              | Maximum | Minimum |                          |                                 |
| Fasting men           |              |  |         |         |  |         |         |                          |                                 |
| Succi                 | 3rd to 12th  | 9.2  | 20.6    | 1.6     | 169  | 242     | 118     | 55                       | 1.00                            |
|                       | 3rd to 12th  | 7.9  | 15.9    | 1.6     | 151  | 214     | 110     | 52                       | 1.00                            |
|                       | 3rd to 12th  | 7.2  | 10.3    | +1.6    |  |         |         |                          | 1.00                            |
|                       | 3rd to 12th  | 7.9  | 17.5    | +1.6    | 114  | 154     | 80      | 69                       | 1.00                            |
|                       | 3rd to 12th  | 8.1  | 14.3    | +4.8    |  |         |         |                          | 1.00                            |
|                       | 3rd to 12th  | 8.4  | 24.6    | 0       |  |         |         |                          | 1.00                            |
|                       | 3rd to 12th  |  |         |         | 129  | 155     | 88      |                          | 1.00                            |
|                       | 3rd to 12th  | 6.2  | 9.7     | 4.2     |  |         |         |                          | 1.00                            |
| Jacques               | 3rd to 12th  | 7.3  | 30.2    | +3.2    |  |         |         |                          | 1.00                            |
| Beanté                | 3rd to 12th  | 6.7  | 12.3    | 0       | 157  | 208     | 127     | 43                       | 1.00                            |
| Schenk                | 3rd to 12th  | 5.5  | 12.5    | 1.8     | 114  | 141     | 78      | 48                       | 1.00                            |
| L                     | 3rd to 12th  | 7.1  | 12.5    | 1.8     | 172  | 195     | 161     | 41                       | 1.00                            |
| Pneumonia             |              |  |         |         |  |         |         |                          |                                 |
| B3                    | Precritical  | 6.7  |         |         | 225  |         |         | 30                       | 0.61                            |
|                       | Postcritical | 10.8                                       |         |         | 226  |         |         | 48                       | 0.62                            |
|                       | Total        | 9.8  | 18.1    | +2.2    | 226  | 338     | 140     | 43                       | 0.62                            |
| B4                    | Precritical  | (+) 2.4                                    |         |         | 435  |         |         | (+) 6                    | 0.68                            |
|                       | Postcritical | 12.3                                       |         |         | 259  |         |         | 47                       | 0.37                            |
|                       | Total        | 9.6  | 33.2    | +4.6    | 291  | 443     | 82      | 33                       | 0.44                            |
| B5 (pleural effusion) | Precritical  | 0.7  |         |         | 290  |         |         | 2                        | 0.46                            |
|                       | Postcritical | 0.0  |         |         | 170  |         |         | 0                        | 0.08                            |
|                       | Total        | 0.3  | 2.8     | +2.8    | 221  | 377     | 38      | 1                        | 0.27                            |
| B6 (with salt)        | Precritical  | 0.1  |         |         | 209  |         |         | 0                        | 0.30                            |
|                       | Postcritical | 1.2  |         |         | 103  |         |         | 12                       | 0.27                            |
|                       | Total        | 0.7  | 1.7     | +1.5    | 156  | 316     | 99      | 4                        | 0.29                            |
| B7 (with salt)        | Precritical  | (+) 10.9                                   |         |         | 152  |         |         | (+) 72                   | 0.49                            |
|                       | Postcritical | (+) 6.9                                    |         |         | 59   |         |         | (+) 117                  | 0.56                            |
|                       | Total        | (+) 9.3                                    | 6.0     | +29.9   | 115  | 286     | 34      | (+) 81                   | 0.52                            |

per cent to as high as 80 per cent of the total water output in these subjects is by way of the skin and lungs. This is correlated of course with the visible sweating observed through much of the period studied. Such visible sweating precludes the possibility of calculating metabolism by the method of Benedict from the loss of weight through skin and lungs. Marked increase in loss of weight by skin and lungs just at the time of the crisis was not an outstanding feature of our cases. The high skin-lung loss of weight in the normal subjects, B8 and B9, was the result of a daily cabinet bath.

It is noteworthy that although the normal subjects B8 and B9 forced drinking of water to the point of discomfort they did not attain the level of water intake and water output reached by the pneumonia patients without any especial persuasion.

#### *Chloride balance*

Our chloride balance studies may be divided into two groups consisting of four patients (B1, B2, B4 and B5) who received no unusual form of treatment, and three patients (B3, B6 and B7) two of whom were given excessive quantities of salt (B6 and B7) and the other (B3) who was given a relatively high caloric diet during the first two days of observation. The chloride in the daily intake of food, water, and enema was measured as well as the chloride output through the urine, feces, sputum, and blood removed for analysis. An allowance of  $\pm 2$  per cent has been made for maximum error.

The attempt was made to collect sweat for chloride analysis. Strips of lint which had been washed free from chloride with distilled water were tied on to the patient's forehead, axillae, and nape of the neck. At frequent intervals the entire body was dried with other strips. However, it proved impossible to obtain satisfactory collections by this method. The best data relative to sweat in pneumonia that was found was that of Schwenkenbecker and Spitta (23). These investigators placed patients on a rubber cloth washed free from chloride and covered them with chloride free linen. After 24 hours they washed the individual and coverings first with water, then with alcohol, and determined the chloride content of the fluid collected. Their conclusion that not more than 1 gram of sodium chloride was excreted daily in the sweat in diseases with profuse sweating such as

pneumonia is consistent with the present observations, for although the collections of sweat in this study were unquestionably incomplete, the maximum daily recovery which was obtained in 19 attempts was 0.27 gram NaCl. To allow for the chloride in the sweat 0.5 gram NaCl, with an allowance for error of  $\pm 0.5$  gram, was arbitrarily added to the measured daily output of chloride.

In table 1 are presented the individual daily chloride balances. The total daily output includes the allowance for sweat. The balance is designated as positive when the intake exceeds the output.

The first group of our cases (B1, B2, B4 and B5) showed daily chloride loss during the precritical period. Even assuming that no chloride was excreted in the sweat, the chloride balance was negative during 8 out of 10 critical and precritical days in these patients. In B3, B6, and B7, on the other hand, there was a daily retention of chloride 15 out of 17 critical and precritical days. The 2 days in which the balance was negative in this group occurred in B3 on the -1 and 0 day at a time when very little food was taken. Case B3 had been given a relatively high caloric diet on the -3 and -2 days of his disease which had to be discontinued on the days following on account of tympanites. After the crisis in the first group and in B3 there was retention of chloride during the first 4 days. During the same period B7 showed a definite loss of chloride and B6, fluctuations in the balance.

This evidence suggests that more chloride is excreted than ingested during the precritical period in lobar pneumonia when the patients receive no forced diet or excessive quantity of salt and that the situation is reversed when larger quantities of chloride are given.

A question which arises in considering the chloride balance is its relation to gain or loss of body water. Associated with a gain or loss of body weight we may properly expect a positive or negative chloride balance respectively. Taking 35 mM per kilogram of body weight as the mean concentration of chloride in the body as a whole, we may approximate an expected chloride balance from a given change in weight. This has been done subject to an allowance of 50 per cent for error and the increase or decrease of chloride so estimated from the change in weight has been designated in the table as the *expected chloride balance*.

The difference between the chloride balance and the expected chloride balance has been indicated merely in a directional manner in the table as the *daily relative chloride balance*. The direction of this quantity has been indicated by + and - signs where the difference has exceeded the sum of the allowances for error. It must be understood that this *relative chloride balance* is an approximation with respect to increase or decrease in the mean chloride concentration in the body as a whole.

B2 and B4 appear to have had definitely a relative chloride loss during the precritical period, B3, B6, and B7, a relative chloride gain, and within the large allowance for error, B1 and B5 had neither clear gain nor loss. During the first 4 days following the crisis a relative gain in chloride was apparent in B1, B3, B4, and B5. In B6 and B7 the diminished chloride intake after the crisis makes it useless to attempt interpretation of that period.

It would appear, therefore, that in the pneumonia patients who received a small intake of chloride per day the mean concentration of chloride in the water of the body either remained unchanged or decreased during the precritical period, whereas after the crisis it increased. In those patients who received a larger intake of chloride there was a gain in the mean concentration of chloride in the body before the crisis and either a gain or loss after the crisis.

*Clinical observations on four lobar pneumonia patients receiving excessive amounts of sodium chloride*

Because of the decreased excretion of chloride in the urine sodium chloride has been given by many therapeutically, often by hypodermoclysis. Haden (24) suggested that sodium chloride should be administered orally to all patients with lobar pneumonia to keep the blood chlorides near the normal level. He gave 74 grams of salt in 4 days to one patient in addition to that taken with food. During these 4 days only 7.23 grams were excreted in the urine. The concentration of chloride in the blood was between 62 and 67 mM per liter followed by a subsequent rise to 82 mM, whether related or not to the crisis is not stated. Later Haden (25) reported the clinical course and the blood and urinary chloride analyses in three cases of lobar pneumonia receiving excessive quantities of salt. Except in one case the excessive salt intake was not clearly associated with a rise in the blood chloride

TABLE 3

*Serum changes in cases on ordinary diet and in cases receiving extra salt*

| Case number | Type of organism      | Day of disease from crisis | Total base            |                                    | Serum chloride | Serum protein               |                                | Serum specific gravity |
|-------------|-----------------------|----------------------------|-----------------------|------------------------------------|----------------|-----------------------------|--------------------------------|------------------------|
|             |                       |                            | Chemically determined | 117 X serum corrected conductivity |                | Serum total nitrogen X 6.25 | Serum protein (refractometric) |                        |
|             |                       |                            | m.Eq per liter        | m.Eq per liter                     | m.Eq per liter | grams per liter             | grams per liter                | 20°C / 20°C            |
| B1          | Pneumococcus Type IV  | -1                         | 136                   | 140                                | 85             | 79                          | 74                             | 1 0232                 |
|             |                       | 0                          | 140                   | 144                                | 85             | 70                          | 76                             | 1 0285                 |
|             |                       | +2                         | 144                   | 146                                | 85             | 72                          | 76                             |                        |
| B2          | Pneumococcus Type III | -3                         | 149                   | 144                                | 92             | 70                          | 65                             |                        |
| B3          | Pneumococcus Type IV  | -3                         | 148                   | 145                                | 87             | 75                          | 76                             |                        |
|             |                       | -2                         | 149                   | 149                                | 91             | 75                          | 80                             |                        |
|             |                       | +1                         | 146                   | 153                                | 89             | 78                          | 88                             |                        |
|             |                       | +3                         | 143                   | 151                                | 94             | 73                          | 74                             |                        |
|             |                       | +6                         |                       |                                    | 99             | 78                          | 81                             |                        |
|             |                       | +8                         | 157                   | 156                                | 97             | 77                          | 78                             |                        |
| B4          | Pneumococcus Type II  | -2                         | 166                   | 154                                | 99             | 67                          | 53                             |                        |
|             |                       | -1                         | 158                   | 146                                | 88             | 65                          | 62                             |                        |
|             |                       | 0                          | 158                   | 142                                | 84             | 65                          | 60                             |                        |
|             |                       | +1                         |                       | 146                                | 86             | 65                          | 62                             |                        |
|             |                       | +3                         | 149                   | 153                                | 93             | 71                          | 86                             |                        |
|             |                       | +5                         | 148                   | 146                                | 95             | 75                          | 78                             |                        |
|             |                       | +7                         |                       | 153                                | 96             | 76                          | 80                             |                        |
|             |                       | +9                         | 148                   | 150                                | 95             | 76                          | 81                             |                        |
| B5          | Pneumococcus Type I   | -3                         |                       | 146                                | 91             | 70                          | 70                             |                        |
|             |                       | -1                         | 146                   | 142                                | 87             | 67                          | 68                             |                        |
|             |                       | +1                         | 138                   | 146                                | 86             | 71                          | 80                             |                        |
| B6*         | Pneumococcus Type IV  | -5                         | 137                   | 151                                | 83             | 63                          | 62                             | 1 0235                 |
|             |                       | -3                         | 143                   | 150                                | 89             | 65                          | 60                             | 1 0234                 |
|             |                       | -2                         | 146                   | 150                                | 95             | 59                          | 56                             | 1 0234                 |
|             |                       | 0                          | 140                   | 149                                | 99             | 64                          | 59                             | 1 0240                 |
|             |                       | +2                         | 149                   | 151                                | 95             | 66                          | 65                             | 1 0239                 |
|             |                       | +5                         | 149                   | 150                                | 91             | 72                          | 71                             | 1 0247                 |

\* Cases receiving salt therapy

TABLE 3—*Concluded*

| Case number | Type of organism      | Day of disease from crisis | Total base             |                                    | Serum chloride         | Serum protein               |                                | Serum specific gravity |
|-------------|-----------------------|----------------------------|------------------------|------------------------------------|------------------------|-----------------------------|--------------------------------|------------------------|
|             |                       |                            | Chemically determined  | 117 X serum corrected conductivity |                        | Serum total nitrogen X 6.25 | Serum protein (refractometric) |                        |
|             |                       |                            | <i>m. Eq per liter</i> | <i>m. Eq per liter</i>             | <i>m. Eq per liter</i> | <i>grams per liter</i>      | <i>grams per liter</i>         | 20°C / 20 C            |
| B7*         | Pneumococcus Type III | -6                         | 142                    | 155                                | 90                     | 74                          | 72                             | 1 0255                 |
|             |                       | -4                         | 143                    | 151                                | 93                     | 80                          | 63                             | 1 0246                 |
|             |                       | -3                         | 143                    | 152                                | 99                     | 66                          | 60                             | 1 0231                 |
|             |                       | -1                         | 161                    | 159                                | 108                    | 64                          | 59                             | 1 0235                 |
|             |                       | +1                         | 155                    | 159                                | 104                    | 64                          | 59                             | 1 0240                 |
|             |                       | +4                         | 153                    | 152                                | 98                     | 69                          | 62                             | 1 0226                 |
| B10*        | Pneumococcus Type IV  | -5                         | 142                    | 146                                | 93                     | 76                          | 76                             | 1 0281                 |
|             |                       | -4                         | 155                    | 153                                | 100                    | 71                          | 73                             | 1 0266                 |
|             |                       | -1                         | 163                    | 169                                | 99                     | 75                          | 84                             | 1 0290                 |
|             |                       | +1                         | 148                    | 151                                | 101                    | 65                          | 65                             | 1 0230                 |
| B11*        |                       | -4                         |                        |                                    | 91                     |                             |                                |                        |
|             |                       | -1                         |                        |                                    | 91                     |                             |                                |                        |
|             |                       | +1                         |                        |                                    | 101                    |                             |                                |                        |

In a previously reported series of cases (26) no outstanding change was found in the serum chloride concentration or clinical course in patients receiving doses of sodium chloride with the food. A statistical analysis of the serum chloride concentrations in those patients showed no significant deviations from the values observed in patients not receiving extra salt. However, in the present study, where considerably more salt was given, increase in the serum chloride concentration is suggested in three cases. In the patients not receiving salt the concentration of serum chloride decreased until the time of crisis in 6 out of 8 patients (cases reported in 1926 (1) and present cases not receiving salt therapy), whereas, in 3 of the 4 cases receiving large quantities of salt the serum chloride concentration progressively increased until the crisis after which salt administration having been stopped, it fell. This is shown in table 3. With the administration of these larger amounts of salt certain clinical effects have been observed which will be described.

In the present group of four patients, each patient received in divided

doses from 15 to 30 grams of sodium chloride per day in the form of a 13.5 per cent sodium chloride solution. The patients objected surprisingly little to these large doses.

### *Abdominal distention*

All four of the patients appeared to have less abdominal distention after salt was given. The abdomen became soft and required less use of stupes or other measures designed to relieve distention. The patients took a slightly higher caloric diet than those not receiving salt, perhaps because of the diminished tympanites. In view of the increased metabolism in pneumonia, it may be accepted that a higher caloric diet than is ordinarily given would be desirable provided the patients could tolerate it. A higher caloric diet without extra salt was tried in B3 but had to be abandoned on account of the abdominal distention which developed.

### *Sputum excretion*

In 14 precritical observations in pneumonia on patients who were receiving no chloride therapy the average amount of sputum collected was 55 grams per day containing 3.7 mM of chloride per day. In 13 precritical observations in patients receiving large quantities of salt, the excretion of sputum averaged 91 grams per day and contained 11.2 mM of chloride per day. In the patients not given salt the chloride concentration in the sputum was 67 mM per kilogram, whereas under larger salt intake it became 124 mM per kilogram, a higher concentration than was present in the serum. The fact that the concentration was higher than in serum may simply be the result of evaporation of water in the respiratory tract.

These facts suggest that the retention of chloride observed during the precritical period when large amounts of salt were given, may in part be explained by the increase of chloride in the pneumonic exudate.

### *Temperature*

The temperature curve in the patients studied appeared to descend in a general way to a lower level when large doses of salt were given. Whether this was incidental can only be decided on the basis of a large series. The temperature instead of varying between 101° and

104° became less fluctuating in type and varied between 99° and 101°. No other drugs than sodium chloride and occasionally codeine sulphate were given to three of the patients, the other, B10, received chloral, barbitol, and sodium bromide on several occasions. In cases B6 and B7 the temperature did not remain continually within the normal range until after the first week following what we considered to be the crisis.

### *Complications*

In three of the four patients postcritical pleural collections and unresolved pneumonic areas were suspected from physical signs and x-ray studies. The patients presented elevations of temperature and moderate leucocytosis after the crisis. The signs were never sufficiently definite to necessitate aspiration and the abnormal chest findings disappeared in the course of about a week. As a result, however, convalescence was protracted by approximately a week.

In studies now in progress there has been substituted for large doses of NaCl a salt mixture containing per diem KCl, 6 grams, NaHCO<sub>3</sub>, 5.9 grams, MgCl<sub>2</sub>, 1.2 grams. One-half of this mixture when added to the ordinary food intake of the patient provides a mean normal salt ration. The caloric value of the diet has also been increased by 500 to 1000 calories with beverages containing 10 per cent glucose.

I wish to express my gratitude to Professor J. Harold Austin for his advice throughout these studies.

### SUMMARY

The total daily ingesta and egesta have been measured before and after the crisis in a series of seven patients suffering with lobar pneumonia. The body weight, nitrogen and chloride balances were determined daily. The loss of weight and inadequacy of the diet have been described and discussed. Partial inanition was an important factor in inducing the observed loss of weight, although additional factors appeared to diminish the rate of loss of weight before the crisis and to increase it after the crisis. The negative nitrogen balance in the pneumonia patients was higher than in fasting individuals.

Patients receiving the usual low caloric diet without extra salt exhibited before the crisis a negative chloride balance, the excretion exceeding the intake. This was true in spite of the extremely reduced excretion in the urine. After the crisis when more food was being taken, chloride excretion in the urine increased, but, nevertheless, there was usually a positive chloride balance. Patients who received a larger intake of chloride during the precritical period either from a more liberal diet or from large doses of salt had precritical retention of chloride.

The chloride balance has been considered also in relation to the change in body weight. In patients receiving a small intake of chloride, the mean concentration of chloride in the body appeared to be decreased before the crisis and increased after the crisis, whereas, in patients receiving a larger intake of chloride (5 to 30 grams NaCl daily) the mean concentration of chloride in the body appeared to be increased before the crisis and decreased after the crisis when the chloride administration had been stopped.

The present studies of the chloride balance do not support the view that pneumonia is characterized by a retention of chloride during the precritical period, but rather that it is characterized during the precritical period by a diminished capacity of the body to conserve chloride on a low intake of chloride and a diminished capacity to excrete chloride on a high intake of chloride. After the crisis the chloride balance becomes restored to normal from whichever deviation had earlier developed.

The clinical picture observed in four patients who received large doses of sodium chloride precritically has been described.

#### BIBLIOGRAPHY\*

- 1 Sunderman, F W, Austun, J H, and Camack, J G, J Clin. Invest., 1926, iii, 37. Studies in Serum Electrolytes. I. Concentration of Electrolytes and Non-electrolytes in the Serum during Lobar Pneumonia.
- 2 Sandelowsky, J, Deut Arch f Klin Med, 1909, xcvi, 445. Blutkonzentration bei Pneumonie.
- 3 Garnier, M, and Sabareanu, G, Compt. Rend Soc. de Biol, 1904, lvi, Ed 1, 1032. Des Modifications du Poids dans la Pneumonie.
- 4 Lussky, H O, and Friedstein, Hugo, Am J Dis. Child., 1920, xix, 337. Water Retention in Pneumonia.

- 5 Leyden, E, Arch f Klin Med, 1869, v, 273 Untersuchungen uber das Fieber
- 6 Hutchison, R, J Path and Bact, 1898, v, 406 Chloride Metabolism in Pneumonia and Acute Fevers
- 7 Garratt, G C, Medico-Chirurgical Trans, 1904, lxxxvii, 163 Observations on Metabolism in the Febrile State in Man
- 8 Von Moraczewski, W, Ztschr f klin Med, 1900, xxxix, 451 Stoffwechsel bei Lungenentzündung Einfluss der Salze auf den selben
- 9 Peabody, F W, J Exper Med, 1913, xvii, 71 Studies in the Inorganic Metabolism in Pneumonia with Especial Reference to Calcium and Magnesium
- 10 Von Hosslin, H, Arch f klin Med, 1908, xciii, 404 Der Kochsalzstoffwechsel bei Pneumonie
- 11 Jarisch, Wien med Jahrbucher 1877, p 39 (Quoted from v Hosslin (13))
- 12 Meillère, Compt rend d Soc Biol, 1901 lvi, 346 (Quoted from v Hosslin (13))
- 13 Terray, v, Ztschr f klin Med, 1894, xxvi, 346 Veränderung des Chlor-Stoffwechsels bei acuten febrilen Erkrankungen
- 14 Prigge, R, Deut Arch f klin Med, 1922, cxxxix, 1 Die Wirkung der intravenösen Zufuhr grosser NaCl-Mengen bei Pneumonie und beim Gesunden
- 15 Holten, C, Arch Int Med, 1926, xxxviii, 489 The Formation of Organic Acids and the Retention of Chlorides in Lobar Pneumonia
- 16 Benedict, F G, and Root, H F, Arch Int Med, 1926, xxxviii, 1 Insensible Perspiration Its Relation to Human Physiology and Pathology
- 17 Bulletin 286, Connecticut Agricultural Experiment Station, part I and II, 1926
- 18 Van Slyke, D D, J Biol Chem, 1923, lviii, 523 The Determination of Chlorides in Blood and Tissues
- 19 DuBois, E F, Basal Metabolism in Health and Disease Philadelphia, 1924, p 332
- 20 Benedict, F G, Pub Carnegie Inst, Washington, 1915, No 203, pp 72, 73, 249 A Study of Prolonged Fasting
- 21 Cook, H W, Bull Johns Hopkins Hosp, 1902, xiii, 307 Nitrogen Excretion in Pneumonia and its Relation to Resolution
- 22 Leyden, E V, and Klemperer, G, Handbuch der Ernährungstherapie, 1904, ii, 345 (Quoted from Lusk's Science of Nutrition, 3rd ed, p 520)
- 23 Schwenkenbecker and Spitta, Arch f exper Path u Pharm, 1907, lvi, 284 Ueber die Ausscheidung von Kochsalz und Stickstoff durch die Haut
- 24 Haden, R L, J Lab and Clin Med, 1925, x, 337 The Relation of the Chloride Metabolism to the Toxemia of Lobar Pneumonia
- 25 Haden, R. L, Am J Med Sci, 1927, clxxiv, 744 The Clinical Significance of the Chloride Metabolism in Lobar Pneumonia

- 26 Sunderman, F W, and Austin, J H., *Atlantic Med J*, 1928, *xxxi*, 294  
Changes in the Serum Salt Concentration in Lobar Pneumonia and Their  
Relation to Therapy

### PROTOCOLS

*Case B1 (Number 962)* Italian, male, age 34, butcher The patient was admitted to the hospital with a consolidation of the left lower lobe Crisis occurred on the 9th day of the disease The sputum yielded a Type IV pneumococcus The leucocyte count was 37,000 with 83 per cent polymorphonuclears on admission Convalescence was uneventful.

*Case B2 (Number 1358)* White, male, age 21, clerk. The patient was admitted to the hospital on the 6th day of the disease with a consolidation of left lower lobe He was cyanotic and breathing rapidly On the 2nd day of hospitalization he became irrational, developed hiccoughs, and died The blood and sputum both yielded a Type III pneumococcus. The leucocyte count on admission was 3,000 but became elevated to 16,000 prior to death

*Case B3 (Number 1208)* Colored, male, age 40, laborer Study of this patient was commenced on the 5th day of a left lower lobar pneumonia. Crisis occurred on the 8th day During the first 2 days of observation, the patient was given a more liberal diet than usual which had to be discontinued on account of the marked abdominal distention. The patient perspired profusely on the day of the crisis The sputum yielded a Type IV pneumococcus The leucocyte count increased progressively from 20,000 on admission to 32,000 at the time of crisis and then fell.

*Case B4 (Number 1046)* Colored, male, age 35, laborer The patient was first studied on the 6th day of his disease at which time he was very ill and had a consolidation of both lower lobes Respirations were voluntarily restricted due to pleural pain Over the left chest pleural and pleuro pericardial frictions were audible Abdominal distention was marked The sputum yielded a Type II pneumococcus Crisis occurred on the 8th day of the disease The leucocyte count was 34,000 2 days after the crisis Convalescence was uneventful.

*Case B5 (Number 1086)* Colored, male, age 11, school boy Study was begun on the 7th day of the disease when the patient had consolidation of the entire left lung The sputum yielded a Type I pneumococcus Crisis occurred on the 10th day of the disease and was followed by the development of an interlobar empyema. A thoracotomy was performed and pus drained The pus contained Type I pneumococcus The highest leucocyte count was 50,000 and was observed at the time of crisis Convalescence lasted 6 weeks

*B6 (Number 1510)* White, male, age 47, longshoreman The patient was first studied on the 3rd day of a left lobar pneumonia The sputum yielded a Type IV pneumococcus Crisis occurred on the 9th day of the disease The temperature, however, did not maintain a postcritical normal level but continued to be elevated (99–101°) during the first week after the crisis Physical signs, x-ray studies, and a leucocytosis of 28,000 at this time suggested the possibility of a pleural collection or unresolved pneumonic areas These chest findings gradually disappeared without treatment Convalescence was protracted

This patient had been given large doses of sodium chloride during the precritical period

*Case B7 (Number 1491)* Colored male, age 57, laborer The patient was first studied on the 3rd day of a left lower lobar pneumonia He was breathing rapidly and appeared toxic Sputum contained a Type III pneumococcus A crisis was not definitely observed but was suggested on the 10th day of the disease Physical signs, x-ray evidence, and a leucocytosis of 15,000 later suggested the possibility of an unresolved pneumonia or pleural collection These signs, however, disappeared Temperature reached the normal level one week after the time that was regarded as the crisis The urine contained on 2 occasions traces of albumin and hyaline and granular casts Following the intake of large doses of sodium chloride during the epicritical period, the patient showed some puffiness especially of the face and a slight edema of the legs

## STUDIES OF SERUM ELECTROLYTES

### V URINARY ELECTROLYTE EXCRETION IN PNEUMONIA<sup>1</sup>

BY J HAROLD AUSTIN AND F WILLIAM SUNDERMAN<sup>2</sup>

*(From the John Herr Musser Department of Research Medicine University of Pennsylvania and the Medical Wards<sup>3</sup> of the Pennsylvania Hospital, Philadelphia)*

(Received for publication October 30, 1928)

In a previous paper (1) one of us has presented studies of weight and nitrogen and chloride balance in pneumonia. It is probable that the changes studied could have been more readily interpreted had we had full information with respect to the other electrolytes as well as with respect to chlorides. To obtain this was not at the time practical. We have, however, data on the urinary excretion of fixed base, sulphate and phosphate from the same subjects over the same period of days in pooled specimens of urine each representing the combined collection of urine from a subject for those consecutive days during which the chloride excretion had been fairly constant. These data are presented and discussed in this paper. Case numbers are the same as in the previous paper.

Total base was measured by Fiske's method (2), inorganic sulphate by Folin's method (3) and phosphate by Briggs' method (4). The figures for phosphate excretion are expressed as m.Eq. which have been calculated by assuming 1.8 m.Eq. per mM. of  $\text{PO}_4$ .

In order that we might have some standard with which to compare the rates of excretion of these electrolytes in our patients with pneumonia we placed two normal subjects for 2 days on a similar dietary intake and had them undergo severe sweating in a cabinet light bath on each of the 2 days. We have made our comparisons with the second day of study of these normal subjects. Furthermore, in order to compare the patients, who differed greatly in size, all excre-

<sup>1</sup> Aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

<sup>2</sup> Robert M. Girvin Fellow in Research Medicine

<sup>3</sup> Service of Dr. George W. Norris

*B6 (Number 1510)* White, male, age 47, longshoreman The patient was first studied on the 3rd day of a left lobar pneumonia The sputum yielded a Type IV pneumococcus Crisis occurred on the 9th day of the disease The temperature, however, did not maintain a postcritical normal level but continued to be elevated (99-101°) during the first week after the crisis Physical signs, x-ray studies, and a leucocytosis of 28,000 at this time suggested the possibility of a pleural collection or unresolved pneumonic areas These chest findings gradually disappeared without treatment Convalescence was protracted

This patient had been given large doses of sodium chloride during the precritical period

*Case B7 (Number 1491)* Colored male, age 57, laborer The patient was first studied on the 3rd day of a left lower lobar pneumonia He was breathing rapidly and appeared toxic Sputum contained a Type III pneumococcus A crisis was not definitely observed but was suggested on the 10th day of the disease Physical signs, x-ray evidence, and a leucocytosis of 15,000 later suggested the possibility of an unresolved pneumonia or pleural collection These signs, however, disappeared Temperature reached the normal level one week after the time that was regarded as the crisis The urine contained on 2 occasions traces of albumin and hyaline and granular casts Following the intake of large doses of sodium chloride during the epicritical period, the patient showed some puffiness especially of the face and a slight edema of the legs

## STUDIES OF SERUM ELECTROLYTES

### V URINARY ELECTROLYTE EXCRETION IN PNEUMONIA<sup>1</sup>

By J HAROLD AUSTIN AND F WILLIAM SUNDERMAN<sup>2</sup>

*(From the John Herr Musser Department of Research Medicine, University of Pennsylvania and the Medical Wards<sup>3</sup> of the Pennsylvania Hospital, Philadelphia)*

(Received for publication October 30, 1928)

In a previous paper (1) one of us has presented studies of weight and nitrogen and chloride balance in pneumonia. It is probable that the changes studied could have been more readily interpreted had we had full information with respect to the other electrolytes as well as with respect to chlorides. To obtain this was not at the time practical. We have, however, data on the urinary excretion of fixed base, sulphate and phosphate from the same subjects over the same period of days in pooled specimens of urine each representing the combined collection of urine from a subject for those consecutive days during which the chloride excretion had been fairly constant. These data are presented and discussed in this paper. Case numbers are the same as in the previous paper.

Total base was measured by Fiske's method (2), inorganic sulphate by Fohn's method (3) and phosphate by Briggs' method (4). The figures for phosphate excretion are expressed as m.Eq. which have been calculated by assuming 1.8 m.Eq. per mM. of  $\text{PO}_4$ .

In order that we might have some standard with which to compare the rates of excretion of these electrolytes in our patients with pneumonia we placed two normal subjects for 2 days on a similar dietary intake and had them undergo severe sweating in a cabinet light bath on each of the 2 days. We have made our comparisons with the second day of study of these normal subjects. Furthermore, in order to compare the patients, who differed greatly in size, all excre-

<sup>1</sup> Aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

<sup>2</sup> Robert M. Gurvin Fellow in Research Medicine.

<sup>3</sup> Service of Dr. George W. Norris.

tion rates have been reduced to constant surface area by multiplying by the ratio of the surface area of the subject to 2.10. The data are given in Table I.

## RESULTS

*Cases not receiving extra salt (B1, B3, B4 and B5)* In the total periods of B1 and B5, in the first period of B3 and in the first two periods of B4 the following features are evident when comparing the excretion rates with those of the second day of the control subjects.

- 1 The total water output by all channels was 35 to 96 per cent above the controls.

- 2 The urinary output however, was not increased in two of the four cases although moderately or markedly increased in the other two.

- 3 In contrast to the low chloride excretion the sum of the measured anions excreted was little reduced or actually increased as compared with the controls. When we consider that Holten (5) has demonstrated considerable increase in organic acid excretion in pneumonia the sum of the anions should probably be still further increased relative to the controls. This deserves emphasis as indicating that the very low chloride excretion in the urine in the precritical period of pneumonia is not necessarily paralleled by low total electrolyte excretion and it suggests the possibility that the low chloride excretion may be in part an adaptation to make way for other anions the excretion of which is perhaps more important.

- 4 The excretion of phosphate was slightly increased in three of the four cases as compared with the controls.

- 5 The excretion of sulphate was strikingly increased in all four as compared with the controls. The ratio of sulphate to nitrogen excreted was in these periods of the patients and in the controls approximately the same and it seems proper to correlate the increased sulphate excretion with the increased protein metabolism.

- 6 The fixed base excretion was greatly reduced ranging from only 20 to 85 per cent of the mean for the two controls. The low base relative to the anion excretion was presumably compensated for by high ammonia and titratable acid in the urine. Our pneumonia patients in the periods under consideration were therefore excreting an excessive amount of certain acid metabolites under the handicap

of limited excretion of fixed base At least one factor in the limitation of fixed base available for excretion was undoubtedly the low base intake of the diet

A consideration of these results suggests that the low chloride excretion in the precritical period of pneumonia is dependent in large

TABLE 1  
*Average excretion per day of measured electrolytes in urine*

| Case number | Days of period | Total water output | Urine  |          |                 |                 |                 |            |                       | Surface area |
|-------------|----------------|--------------------|--------|----------|-----------------|-----------------|-----------------|------------|-----------------------|--------------|
|             |                |                    | Water  | Chloride | SO <sub>4</sub> | PO <sub>4</sub> | Measured anions | Fixed base | SO <sub>4</sub> 3.61N |              |
|             |                | liters             | liters | m. Eq    | m Eq            | m Eq            | m. Eq           | m Eq       |                       | sq m.        |
| B1          | -1 +1          | 5 3                | 1 26   | 0        | 72              | 43              | 115             | 22         | 0 82                  | 1 68         |
| B3          | -3, +1         | 4 3                | 1 35   | 7        | 78              | 68              | 153             | 86         | 0 89                  | 2 40         |
|             | +2 +7          | 4 1                | 2 08   | 88       | 64              | 54              | 206             | 152        | 1 01                  |              |
| B4          | -2, +3         | 5 4                | 2 01   | 30       | 85              | 74              | 194             | 135        | 0 88                  | 1 91         |
|             | +4 +6          | 4 4                | 2 48   | 37       | 81              | 81              | 142             | 142        |                       |              |
|             | +7, +8         | 3 8                | 3 03   | 176      | 109             | 79              | 364             | 282        | 1 25                  |              |
| B5          | -3, +3         | 6 3                | 3 66   | 29       | 68              | 34              | 131             | 60         | 0 81                  | 1 02         |
| B6          | -5, -4         | 5 2                | 2 01   | 34       | 154             | 63              | 251             | 62         | 1 02                  | 1 68         |
|             | -3, +4         | 4 8                | 2 31   | 338      | 46              | 52              | 436             | 298        |                       |              |
| B7          | -6, -5         | 4 0                | 1 23   | 3        | 61              | 31              | 95              | 52         | 0 94                  | 1 87         |
|             | -4, -1         | 3 8                | 2 05   | 83       | 47              | 34              | 146             | 80         |                       |              |
|             | 0, +1          | 2 4                | 1 12   | 48       |                 |                 |                 | 75         |                       |              |
|             | +2, +3         | 2 3                | 1 19   | 45       |                 |                 |                 | 83         |                       |              |
| B8          | 2              | 3 1                | 1 09   | 61       | 43              | 49              | 153             | 117        | 0 89                  | 2 05         |
| B9          | 2              | 3 3                | 1 62   | 58       | 40              | 50              | 148             | 129        | 0 87                  | 2 17         |

\* Estimated as 1 8 m Eq per mM

measure on low chloride intake and perhaps in part upon the need for excretion of excessive amounts of sulphate, phosphate, and organic acid under the handicap of little fixed base available for excretion. It is possible, furthermore, that this demand for fixed base for excretion is a factor in the low base concentration in the serum in the precritical period of pneumonia. Correlated with this would be a

tendency for lowering of the serum chloride. Furthermore, in the need for transporting the increased amounts of sulphate, organic acids, and phosphate from the tissues to the kidneys we have perhaps a reason, although not a mechanism, for the disproportionate lowering of the serum chloride.

In the two patients B3 and B4, studied through more than one period the following features are evident in the final period.

7 There was a diminution in the high total output of water associated with increase in the urine and necessarily diminished loss of water by skin and lungs.

8 The fixed base excretion was increased.

9 The ratio of sulphate to nitrogen increased.

10 The chloride excretion increased in correlation we believe, with the increased intake of chloride and fixed base consequent upon the more liberal food intake.

*Cases receiving extra salt (B6 and B7)* As compared with the patients receiving no extra salt these two subjects exhibited high excretion of fixed base and chloride. In B7 the excretion of chloride was much below the intake and this was associated with marked retention of water and eventually with visible edema. That a tendency to chloride retention characterizes the behavior of patients with pneumonia when given NaCl freely was pointed out in the previous paper (1).

#### SUMMARY

The low chloride excretion observed in the urine in the precritical period in patients on the usual low diet not receiving salt was associated with low urinary excretion of fixed base but with very high excretion of sulphate and phosphate so that the total anion excretion in the urine was not far from that of normal subjects on a similar diet. The high sulphate excretion was proportional to the high nitrogen excretion and is probably to be correlated with the tissue catabolism.

#### BIBLIOGRAPHY

- 1 Sunderman, F. W., J. Clin. Invest., 1929, vii, 313. Studies of Serum Electrolytes. IV. The Chloride and Nitrogen Balances and Weight Changes in Pneumonia.

- 2 Fiske, C H , J Biol. Chem , 1922, II, 55 A Method for the Estimation of Total Base in Urine.
- 3 Folin, Otto, Laboratory Manual of Biological Chemistry, New York, 1922, 3rd ed.
- 4 Briggs, A. P , J Biol. Chem., 1922, III, 13 A Modification of the Bell Doisy Phosphate Method
- 5 Holten, C , Arch. Int Med , 1926, xxxviii, 489 The Formation of Organic Acids and the Retention of Chlorides in Lobar Pneumonia



# THE EFFECT OF EXERCISE ON THE SIZE OF NORMAL HEARTS AND OF ENLARGED HEARTS OF DOGS

By HAROLD J STEWART

*(From the Hospital of the Rockefeller Institute for Medical Research New York)*

(Received for publication February 6 1929)

Although the changes in the heart produced by exercise have long been made the subject of investigation by clinicians and physiologists, the question has not yet been settled. The earliest view, formulated from clinical experience, was that when the heart was subjected to exertion it dilated. This opinion was derived from estimations of changes in the borders of the heart on percussion (T Schott (1890) and Williams and Arnold (1899)). T Schott (1897) was the first investigator to employ x ray examination in studying this problem. The data he collected appeared to support his view that dilatation of the heart occurred. Moritz (1908) in 1908 first demonstrated by means of orthodiagrams that acute cardiac enlargement did not follow over-exertion. Indeed, on the contrary, he found that in certain instances both in normal and pathologic hearts contraction took place. Since then, studies have been made of the effect of many varieties of exertion (swimming, rowing, running in marathon races) on the heart of man, from the point of view not only of discovering whether the heart reacts to acute exertion by dilating or contracting, but also of learning whether hypertrophy is necessarily a consequence of athletic training. More and more, the evidence which has been accumulated seems to indicate that dilatation does not occur as a consequence of acute exertion, but that on the contrary contraction in size takes place. Recently Gordon and Strong (1923) have studied in rabbits the effect of vigorous exercise on the size of the normal and abnormal heart. The hearts designated as abnormal were those in which enlargement resulted from repeated injections of spartein sulphate. According to these authors myocarditis was the resulting pathologic lesion. They found that the effect of exercise was the



same in both instances a decrease in size of the hearts always occurred

We have had an opportunity also to investigate this subject For several years we have been engaged in attempting to find a method which would establish a state in dogs comparable to heart failure in man One of the methods has consisted in the attempt to bring about this state by rendering the mitral valve insufficient We have for this reason in our possession a number of dogs in which defects of the mitral valve have been made by operation and in which enlarged hearts have in consequence developed They show no signs however of heart failure It has been in connection with the study of the circulation of these dogs that we have investigated the response of the hearts to exercise In them we are able to estimate the influence that valvular defect alone (presumably without disease of the muscle) exerts on the reaction of the heart to exertion The form of exercise which we chose was running on a treadmill We took the precaution of studying the effect of the same form of exertion on the hearts of normal dogs These experiments form the subject of this paper

#### MATERIAL

The subjects of certain experiments, as has been stated, were normal intact dogs The dogs which were the subjects of other experiments were those which had been operated on 2 to 3½ years ago Evidence of the lesions which were then created still existed at the time of the present experiments (table 1) Complete data concerning the operations will be published later (Stewart) A brief description only of the method used in operating on the valves need be given Under ether anesthesia and under aseptic conditions the left auricular appendage was exposed and incised A cardioscope<sup>1</sup> was then inserted through this opening By manipulation of the knife attached to the cardioscope the leaflets of the

---

<sup>1</sup> The cardioscope which we used was designed with the assistance of Mr R Wappler, and was made for us by the Wappler Electric Company, Long Island City, New York. The idea of cutting the valves of the heart under direct vision was suggested to us by the preliminary report of Allen and Graham (1922) As complete data for the construction of their instrument was not available at the time, we devised this new instrument. The optical system is similar to that used in cystoscopes. We are much indebted to Doctors Graham and Allen for valuable aid in learning their methods and desire to express our thanks to them for their courtesy



1a



1b



1c

FIG. 1 In this figure are reproduced X-ray photographs showing the effect of exercise on the size of the normal heart. Photograph 1a was taken before and 1b after running. 1c was taken after the dog had rested for 1 hour. Photo

same in both instances a decrease in size of the hearts always occurred

We have had an opportunity also to investigate this subject. For several years we have been engaged in attempting to find a method which would establish a state in dogs comparable to heart failure in man. One of the methods has consisted in the attempt to bring about this state by rendering the mitral valve insufficient. We have for this reason in our possession a number of dogs in which defects of the mitral valve have been made by operation and in which enlarged hearts have in consequence developed. They show no signs however of heart failure. It has been in connection with the study of the circulation of these dogs that we have investigated the response of the hearts to exercise. In them we are able to estimate the influence that valvular defect alone (presumably without disease of the muscle) exerts on the reaction of the heart to exertion. The form of exercise which we chose was running on a treadmill. We took the precaution of studying the effect of the same form of exertion on the hearts of normal dogs. These experiments form the subject of this paper.

#### MATERIAL

The subjects of certain experiments, as has been stated, were normal intact dogs. The dogs which were the subjects of other experiments were those which had been operated on 2 to 3½ years ago. Evidence of the lesions which were then created still existed at the time of the present experiments (table 1). Complete data concerning the operations will be published later (Stewart). A brief description only of the method used in operating on the valves need be given. Under ether anesthesia and under aseptic conditions the left auricular appendage was exposed and incised. A cardioscope<sup>1</sup> was then inserted through this opening. By manipulation of the knife attached to the cardioscope the leaflets of the

---

<sup>1</sup> The cardioscope which we used was designed with the assistance of Mr. R. Wappler, and was made for us by the Wappler Electric Company, Long Island City, New York. The idea of cutting the valves of the heart under direct vision was suggested to us by the preliminary report of Allen and Graham (1922). As complete data for the construction of their instrument was not available at the time, we devised this new instrument. The optical system is similar to that used in cystoscopes. We are much indebted to Doctors Graham and Allen for valuable aid in learning their methods and desire to express our thanks to them for their courtesy.

TABLE 1

*Effect of exercise on the size of the heart of normal dogs*

| Dog number | Date             | Weight | Cardiac area | Cardiac area per cent of initial | Decrease in cardiac area | Heart rate | Duration of exercise | Distance run | Time with reference to running |
|------------|------------------|--------|--------------|----------------------------------|--------------------------|------------|----------------------|--------------|--------------------------------|
|            |                  | kgrm   | sq cm        | per cent                         | per cent                 | per minute | minutes              | miles        |                                |
| 235        | March 22, 1927   | 15 2   | 60 4         | 100 0                            |                          | 96         |                      |              | Before                         |
|            |                  |        | 57 3         | 94 8                             | 5 2                      | 120        | 45                   | 0 9          | Immediately after              |
|            |                  |        | 56 1         | 92 8                             | 7 2                      | 128        |                      |              | One hour after                 |
| 250        | October 26, 1927 | 10 5   | 35 8         | 100 0                            |                          | 120        |                      |              | Before                         |
|            |                  |        | 34 4         | 96 1                             | 3 9                      | 110        | 60                   | 0 8          | Immediately after              |
|            |                  |        | 34 9         | 97 5                             | 2 5                      | 130        |                      |              | One hour after                 |
|            | October 27, 1927 | 11 0   | 40 8         | 100 0                            |                          | 120        |                      |              | Before                         |
|            |                  |        | 38 9         | 95 4                             | 4 6                      | 90         | 60                   | 1 1          | Immediately after              |
|            |                  |        | 37 2         | 91 2                             | 8 8                      | 120        |                      |              | One hour after                 |
|            | October 28, 1927 | 11 6   | 36 3         | 100 0                            |                          | 120        |                      |              | Before                         |
|            |                  |        | 35 6         | 98 0                             | 2 0                      | 90         | 60                   | 1 3          | Immediately after              |
|            |                  |        | 34 0         | 93 7                             | 6 3                      | 120        |                      |              | One hour after                 |
|            | October 29, 1927 | 10 3   | 37 9         | 100 0                            |                          | 85         |                      |              | Before                         |
|            |                  |        | 35 7         | 94 2                             | 5 8                      | 85         | 45                   | *            | Immediately after              |
|            |                  |        | 35 6         | 94 0                             | 6 0                      | 85         |                      |              | One hour after                 |
| 251        | October 27, 1927 | 12 7   | 48 8         | 100 0                            |                          | 110        |                      |              | Before                         |
|            |                  |        | 46 1         | 94 5                             | 5 5                      | 90         | 35                   | 0 4          | Immediately after              |
|            |                  |        | 46 1         | 94 5                             | 5 5                      | 115        |                      |              | One hour after                 |

\* Speedometer not working

TABLE 2

*Enlargement of the heart following induction of artificial mitral insufficiency in dogs*

| Dog number | Area of heart before operation | Area of heart after operation | Time since operation | Increase in area of heart | Presence of murmur at time of running |
|------------|--------------------------------|-------------------------------|----------------------|---------------------------|---------------------------------------|
|            | sq cm.                         | sq cm.                        | years                | per cent                  |                                       |
| 158        | 46 4                           | 84 4                          | 3 $\frac{1}{2}$      | 81 8                      | +                                     |
| 161        | 46 0                           | 72 3                          | 2 $\frac{1}{2}$      | 57 2                      | +                                     |
| 171        | 50 3                           | 68 9                          | 2                    | 37 7                      | +                                     |
| 153        | 56 2                           | 68 2                          | 2 $\frac{1}{2}$      | 21 3                      | 0                                     |
| 90         | 43 0                           | 42 6                          | 3 $\frac{1}{2}$      | 0 0                       | 0                                     |

mitral valve were brought into view. The leaflets could then be cut under direct vision. Development of a marked systolic thrill was regarded as evidence that the operation had succeeded. The dogs completely recovered within 10 days to 2 weeks. The hearts began to increase in size after varying intervals of time. A loud systolic murmur persisted in each of the dogs (see exceptions, table 2). The dogs studied for the present purpose were in good health. They were trained to laboratory procedures and were not disturbed by them.

#### METHODS

The dogs were first trained to run on a treadmill. Certain dogs ran readily, others could not be induced to run and were not studied. After the preliminary training, the effect of running on the size of the heart was investigated by means of x ray photographs of the heart. The x ray photographs were made and measured according to the method described by Stewart (1927) for obtaining photographs of the hearts of dogs under uniform conditions. The anticathode was placed at a distance of 2 meters from the photographic film. A suture was inserted in the skin in the mid line of the anterior chest wall at the level of the heart. The anti-cathode was always centered on this point before plates were exposed. Three x ray photographs were made during each session: the first, before the dog began running, the second, immediately after the dog had stopped running, and the third, after the dog had rested for one hour. Since the treadmill was not driven by a motor, but by the dogs themselves, they ran only as long as they did so voluntarily. They ran quite steadily for 25 to 60 minutes. The second photographs were made as soon as it was evident that the dogs did not wish to run longer. The tread was placed at an angle of 19 degrees with the horizontal and a speedometer recorded the number of revolutions from which the distance was calculated. Since the treadmill was in the x ray room only a few seconds were required to transfer the dog from it to the dog board under the x ray tube. The x ray photographs were taken within 2 to 3 minutes after the dogs had stopped running. The rate of the heart was counted at the apex for one minute immediately after the photographs were taken. The observations were usually repeated several times in each dog.

The exposures were sufficiently long to photograph the diastolic heart shadow in those photographs in which both systolic and diastolic positions of the heart could be identified, the diastolic area was the one measured.

#### OBSERVATIONS

*Effect of exercise on the size of the heart in normal dogs.* In dog 235 the initial cardiac area was 60.4 sq cm (table 2, fig 1). It decreased to 57.3 sq cm after the dog had been running for 45 minutes. After resting 1 hour it decreased still further to 56.1 sq cm. There was

TABLE 3

*The effect of exercise on the size of enlarged hearts in which systolic murmurs were still present*

| Dog number | Date              | Weight | Cardiac area | Cardiac area per cent of initial | Decrease in cardiac area | Heart rate | Duration of exercise | Distance run | Time with reference to running |
|------------|-------------------|--------|--------------|----------------------------------|--------------------------|------------|----------------------|--------------|--------------------------------|
|            |                   |        |              |                                  |                          |            |                      |              |                                |
|            |                   | kgm    | sq cm        | per cent                         | per cent                 | per minute | minutes              | miles        |                                |
| 158        | February 10, 1927 | 20 6   | 82 1         | 100 0                            |                          | 130        |                      |              | Before                         |
|            |                   |        | 77 2         | 94 0                             | 6 0                      | 148        | 60                   | 0 6          | Immediately after              |
|            |                   |        | 81 9         | 99 7                             | 0 3                      | 136        |                      |              | One hour after                 |
|            | March 4, 1927     | 22 3   | 87 5         | 100 0                            |                          | 148        |                      |              | Before                         |
|            |                   |        | 80 4         | 91 8                             | 8 2                      | 132        | 38                   | 0 4          | Immediately after              |
|            |                   |        | 82 8         | 94 7                             | 5 3                      | 150        |                      |              | One hour after                 |
|            | October 13, 1927  | 19 9   | 84 4         | 100 0                            |                          | 90         |                      |              | Before                         |
|            |                   |        | 79 1         | 93 7                             | 6 3                      | 120        | 40                   | 0 5          | Immediately after              |
|            |                   |        | 80 9         | 95 8                             | 4 2                      | 114        |                      |              | One hour after                 |
|            | October 15, 1927  | 20 1   | 87 2         | 100 0                            |                          | 130        |                      |              | Before                         |
|            |                   |        | 79 9         | 91 6                             | 8 4                      | 110        | 57                   | 0 6          | Immediately after              |
|            |                   |        | 83 7         | 96 0                             | 4 0                      | 130        |                      |              | One hour after                 |
| 161        | January 28, 1927  | 16 0   | 72 3         | 100 0                            |                          | 160        |                      |              | Before                         |
|            |                   |        | 65 9         | 91 1                             | 8 9                      | 142        | 38                   | 0 3          | Immediately after              |
|            |                   |        | 71 0         | 98 2                             | 1 8                      | 144        |                      |              | One hour after                 |
|            | February 9, 1927  | 16 8   | 73 6         | 100 0                            |                          | 122        |                      |              | Before                         |
|            |                   |        | 68 3         | 94 1                             | 5 9                      | 142        | 25                   |              | Immediately after              |
|            |                   |        | 68 5         | 94 4                             | 5 6                      | 134        | 31                   | 0 2          | Immediately after              |
|            |                   |        | 74 1         | 100 6                            | 0 6                      |            |                      |              | One hour after                 |
|            | October 13, 1927  | 14 5   | 72 0         | 100 0                            |                          | 104        |                      |              | Before                         |
|            |                   |        | 68 3         | 94 8                             | 5 2                      | 104        | 30                   | 0 3          | Immediately after              |
|            |                   |        | 71 2         | 98 8                             | 1 2                      | 120        |                      |              | One hour after                 |
| 171        | March 14, 1927    | 20 5   | 68 9         | 100 0                            |                          | 140        |                      |              | Before                         |
|            |                   |        | 66 9         | 97 1                             | 2 9                      | 130        | 60                   | 0 8          | Immediately after              |
|            |                   |        | 62 8         | 91 2                             | 8 8                      | 148        |                      |              | One hour after                 |
|            | March 15, 1927    | 21 3   | 64 5         | 100 0                            |                          | 140        |                      |              | Before                         |
|            |                   |        | 60 5         | 93 8                             | 6 2                      | 142        | 44                   | 0 8          | Immediately after              |
|            |                   |        | 59 0         | 91 5                             | 8 5                      | 140        |                      |              | One hour after                 |



2c

2b

2a

FIG 2 In this figure are reproduced x ray photographs which show the effect of exercise on the size of the enlarged heart Photograph 2a was taken before and 2b after running 2c was made after the dog rested for 1 hour

accordingly a fall to 94.8 per cent of the initial size followed by a further decrease to 92.8 per cent of the initial size one hour after exercise

There are 4 observations on dog 250 and one on dog 251 (Table 2). The results in all instances are similar to the one just reported. The size of the hearts decreased 3.9 to 5.8 per cent immediately after running, but decreased still further 6.0 to 8.8 per cent 1 hour later. In not a single instance did the size of the heart become greater than it had been in the beginning.

*Effect of exercise on the size of enlarged hearts in which systolic murmurs are present.* Mitral insufficiency was created in dog 158 on December 2, 1924, 3½ years ago. During this time the area of the heart increased 81.8 per cent from 46.4 sq cm to 84.4 sq cm (table 2). This was the area on October 13, 1927 (table 3, fig. 2). After running 40 minutes on this day it decreased to 79.1 sq cm, 93.7 per cent of the value before exercise. One hour later it was 80.9 sq cm, 95.8 per cent of what it was before the start. There was then on this occasion a decrease of 6.3 per cent in the size of the heart after exercise. The dog ran on three other occasions. On these also the cardiac area decreased, the maximum decrease varying between 6.0 and 8.4 per cent.

Mitral insufficiency was created in dog 161 on December 11, 1924, 2½ years ago. During this time the area of the heart increased 57.2 per cent from 46.0 sq cm to 72.3 sq cm (table 2). This was the cardiac area on January 28, 1927 (table 3). After running 38 minutes on this day it was 65.9 sq cm, that is to say, it had diminished 8.9 per cent. One hour later it was 71.0 sq cm, approximating the size it was before the start. The dog ran on two other occasions. On these also a decrease in size of the heart occurred. The maximum decrease amounted to 5.9 per cent and 5.2 per cent respectively.

Mitral insufficiency was created in dog 171 on April 22, 1925, 2 years ago. During this time the heart had enlarged 37.7 per cent from 50.3 sq cm to 68.9 sq cm (table 2). He ran on the treadmill on two occasions. The decreases immediately after running were 2.9 and 6.2 per cent respectively and were 8.8 and 8.5 per cent respectively one hour later (table 3).

There are observations therefore on 3 dogs in which the heart was

enlarged and in which there was still evidence that defects of the mitral valve (murmurs) were still present. In every instance the size of the heart decreased following the exercise of running on a treadmill

*Effect of exercise on the size of the heart in dogs subjected to operation but in which there was no longer evidence that valvular defect was present*  
Mitral insufficiency was created in dog 153 on November 20, 1924,

TABLE 4

*Effect of exercise on the size of the hearts of dogs subjected to operation but in which systolic murmurs were no longer present*

| Dog number | Date             | Weight | Cardiac area | Cardiac area per cent of initial | Decrease in cardiac area | Heart rate | Duration of exercise | Distance run | Time with reference to running |
|------------|------------------|--------|--------------|----------------------------------|--------------------------|------------|----------------------|--------------|--------------------------------|
|            |                  |        |              |                                  |                          |            |                      |              |                                |
| 153        | January 18 1927  | 23 0   | 69 6         | 100 0                            |                          |            |                      |              | Before                         |
|            |                  |        | 66 7         | 95 8                             | 4 2                      |            | 42                   | 0 6          | Immediately after              |
|            |                  |        | 62 9         | 90 4                             | 9 6                      |            |                      |              | 1 hour after                   |
|            | January 22, 1927 | 23 0   | 67 8         | 100 0                            |                          | 182        |                      |              | Before                         |
|            |                  |        | 60 9         | 89 0                             | 11 0                     | 145        | 55                   | 0 6          | Immediately after              |
|            |                  |        | 67 9         | 100 0                            |                          | 170        |                      |              | 1 hour after                   |
|            | January 25, 1927 | 23 0   | 68 2         | 100 0                            |                          | 165        |                      |              | Before                         |
|            |                  |        | 62 5         | 91 5                             | 8 5                      | 170        | 50                   | 0 4          | Immediately after              |
|            |                  |        | 67 5         | 99 0                             | 1 0                      | 155        |                      |              | 1 hour after                   |
| 90         | April 15, 1927   | 11 5   | 42 6         | 100 0                            |                          | 130        |                      |              | Before                         |
|            |                  |        | 40 2         | 94 3                             | 5 7                      | 120        | 38                   | 0 8          | Immediately after              |
|            |                  |        | 42 6         | 100 0                            |                          | 112        |                      |              | 1 hour after                   |

2½ years ago During this time the size of the heart increased 21 3 per cent, from 56 2 sq cm to 68 2 sq cm (table 2) A systolic murmur was heard for some months after operation but later it disappeared It was not heard at the time of these experiments The dog ran on three occasions On each occasion the heart was smaller after running than it had been in the beginning (table 4) It decreased 8.5 per cent on the first, 11 per cent on the second and 4 2 per cent on the third occasion One hour later it had nearly regained its

initial size on the first, it had returned to its initial size on the second, and decreased still further (9.6 per cent) on the third occasion

Mitral insufficiency was created in dog 90 on February 28, 1924. A soft systolic murmur was heard for some months after operation but later it disappeared. Three and one sixth years after operation the area of the heart was approximately the same as before operation (0.8 per cent decrease) (table 2). The area of the heart was 42.6 sq cm on April 15, 1927 (table 4). After running on the treadmill for 38 minutes it was 40.2 sq cm, that is to say, a decrease of 5.7 per cent had occurred. The initial size was regained one hour later.

In one dog (3 occasions) then the subject of an artificially enlarged heart and in a second in which there was no enlargement, the hearts decreased in size after running on a treadmill. The maximum decrease was 11 per cent. A systolic murmur was not heard in either case.

*Effect of exercise on the heart rate.* The heart rate decreased after running in 2 normal dogs (dog 250, 3 occasions and dog 251, once) (table 2), unchanged once (dog 250) and increased once (dog 235). In the case of the dogs in which the hearts were enlarged, the rate sometimes decreased (dog 158 twice, dog 161 once, dog 171 once, dog 90 once, and dog 153 once), sometimes increased (dog 158 twice, and dog 161 once) and was sometimes unchanged (dog 161 once, dog 171 once, and dog 153 once). It is clear that the same effect was not always observed even in the same dog.

#### DISCUSSION

We have presented data showing that the hearts of normal dogs decrease in size following exercise. Decrease in size also occurred in hearts that were enlarged, whether the valve defect (murmur) was or was not still present. The effect in dogs in which the hearts were enlarged was the same as in the normal hearts, the dogs were all in good health and exhibited no evidence of heart failure. Although the mitral valves had been subjected to damage and the hearts had enlarged in consequence of this injury, there had occurred no disease of the heart muscle so far as is known. Although the decrease in size was small, 3.9 to 11 per cent, it is nevertheless significant and it occurred consistently. Enlargement never was observed. In the hour after exercise the hearts sometimes decreased still further in size.

(if the decrease in the first instance was small), sometimes remained unchanged and at other times regained their initial size. We have no explanation to offer for this difference. The changes which we observed in dogs were not as great as those observed in rabbits by Gordon and Strong (1923). The difference in the results obtained is probably due to the fact that the rabbits ran until completely exhausted, while our dogs ran only as long as they did so voluntarily. The distance which the dogs ran may appear to be small. The average distance was 0.6 mile, but the inclination of the tread (19°) necessitated a vertical ascent of 1000 ft. in this distance.

We cannot be certain of the factors responsible for the occurrence of the decrease in size of the hearts of these dogs after exercise. It was observed also to accompany regular tachycardia (Stewart and Crawford, 1927). Decreased filling of the heart in the shortened diastole may be one of the factors. In the case of these experiments however tachycardia was not a factor, since the ventricular rate exhibited no consistent change, remaining unchanged or becoming either slower or faster than the initial rate. Increase in size of the vascular bed may of course have taken place due to the opening of channels which were hitherto closed, for exercise may be believed to be the occasion for the opening of a greater number of capillaries in the muscles and skin than when the animals are in a resting state. The blood would then be drained away from the heart.

Both Meek and Eyster (1922) and Stewart (Stewart, a) have shown that the size of the heart decreases when the volume of circulating blood is diminished. But decrease in volume of blood was in all probability not a factor in these cases since Hastings (1921) has shown an increase following exercise in the hemoglobin content (expressed as oxygen capacity) of the blood and Broun (1922) has demonstrated an actual increase in the total blood volume.

In another connection also there is similarity in behavior of large and small hearts. The same dogs which served in these experiments were utilized also in studying the effect of digitalis. In both groups decrease in cardiac size, decrease in cardiac output and increase in ventricular excursion followed the administration of this drug (Cohn and Stewart, 1928a and 1928b).

It is not certain how far an application can be made of the data of

these experiments to cases of heart disease in man. The analogy is not close enough to warrant the conclusion that acute exercise is not harmful to patients suffering from valvular disease when they exhibit no signs of heart failure. It is infrequent that one finds in patients the conditions which were present in these experiments, that is to say, valvular insufficiency and cardiac enlargement without concomitant disease of the myocardium.

#### SUMMARY

The effect of running on a treadmill on the size of the hearts of dogs has been studied. It was found that the size of both normal and enlarged hearts always decreased.

#### CONCLUSIONS

Dilatation of the heart does not occur in normal dogs following voluntary exercise. On the contrary, the size of the heart decreases. When dogs in which the heart is enlarged in consequence of artificially created valvular defects, but in which there is presumably no myocardial disease, and in which there are no signs of heart failure, are subjected to exercise, the size of the heart likewise decreases.

#### BIBLIOGRAPHY

- Allen, D. F., and Graham, E. A., *J. Am. Med. Assoc.*, 1922, lxxix, 1028. Intra-cardiac Surgery—A New Method. Preliminary Report.
- Broun, G. O., *J. Exper. Med.*, 1922, xxxvi, 481. Blood Destruction During Exercise. I. Blood Changes Occurring in the Course of a Single Day of Exercise.
- Cohn, A. E., and Stewart, H. J. (1928a), *J. Clin. Invest.*, 1928, vi, 79. The Relation Between Cardiac Size and Cardiac Output per Minute Following the Administration of Digitalis in Dogs in Which the Heart is Enlarged.
- Cohn, A. E., and Stewart, H. J. (1928b), *J. Clin. Invest.*, 1928, vi, 53. The Relation between Cardiac Size and Cardiac Output per Minute Following the Administration of Digitalis in Normal Dogs.
- Gordon, B., and Strong, G. F., *Arch. Int. Med.*, 1923, xxxii, 517. Studies on the Rabbit's Heart. II. Effect of Vigorous Exercise on the Size of the Normal and the Abnormal Heart.
- Hastings, A. B., *Public Health Bulletin No. 117*. May, 1921. Treasury Department U. S. Public Health Service, Wash., Govt. Printing Office, 1921. The Physiology of Fatigue. Physico-chemical Manifestations of Fatigue in the Blood.

- Meek, W J, and Eyster J A. E, *Am. J Physiol.*, 1922, lxi 186 The Effect of Plethora and Variations in Venous Pressure on Diastolic Size and Output of the Heart
- Moritz, F, *Münch med Wchnschr*, 1908, iv, 713 Ueber funktionelle Verkleinerung des Herzens
- Moritz, F, *Münch med Wchnschr*, 1908, iv, 1331 Zur Frage von akuten Dilatation des Herzens durch Ueberanstrengung
- Schott, T, *Verhand. d Congr f inn Med.* 1890 ix, 448 Zur acuten Ueberanstrengung des Herzens und deren Behandlung
- Schott, T, *Deutsch med Wchnschr*, 1897, xxvi, 495 III Experimente mit Röntgenstrahlen über acute Herzüberanstrengung
- Stewart, H. J Experiments to be published
- Stewart, H J, *Arch Pathol*, 1929, vii, 767 (a) A Study of Certain Effects Occasioned in Dogs by Diphtheria Toxin II An Analysis of the Mechanisms possibly Responsible for the Alterations of the Heart
- Stewart, H J, *J Clin Invest.*, 1927, iii, 475 A Technique for measuring X ray Photographs of the Cardiac Areas of Dogs
- Stewart, H J and Crawford, J Hamilton, *J Clin. Invest*, 1927, iii, 483 The Effect of Regular and Irregular Tachycardias on the Size of the Heart.
- Williams, H, and Arnold H D, *Phila Med J*, 1899, ii, 1233 The Effects of Violent and Prolonged Muscular Exercise upon the Heart.



# METABOLISM OF CHLORIDE AND TOTAL FIXED BASE IN PNEUMONIA AND THE RELATION TO SALT AND WATER RETENTION

BY T S WILDER AND T G H. DRAKE

*(From the Department of Pediatrics Harvard Medical School, and the Research Laboratory of the Children's Hospital, Boston)*

(Received for publication March 18, 1929)

Although much has been written regarding an unusual behavior of chloride and certain other ions in pneumonia, the literature fails to supply a clear cut conception of the sequence of events in the metabolism of acid and base in this disease. Largely because investigations have tended to consider only a few factors at a time it is difficult to evaluate their relationship to other elements in the situation. Several findings have, however, been thoroughly established, and it is the object of this paper to attempt an explanation of them in terms of certain accompanying circumstances. An underlying variable, the effect of which we have particularly studied, is the level of salt and water intake.

Peabody (1) (2), in his papers of 1912 and 1913, summarized the previous literature and reported the observations that most patients with pneumonia have low serum and urinary chlorides, and a high urine ammonia. He reported also a decreased elimination of Na, Ca, and Cl, but a normal or increased excretion of K and Mg, inferring therefrom a retention of the former ions but not of the latter. By analysis of tissues, Peabody went on to show that there is nowhere an accumulation of the "retained" ions, which led him to believe that these must be spread diffusely through the body.

McLean (3) in 1915 confirmed Peabody's findings and noted also that the low serum chloride found in pneumonia returned to normal before the low urinary chloride showed a similar change.

In 1916 Maver and Schwartz (4) observed the presence of pitting edema in some cases of pneumonia and not in others, but felt that their

## EXPERIMENTAL

Patient W H was studied for the 3 days preceding and the 6 days succeeding his crisis. During the first 3 days he gained nearly 2 pounds in weight, which same amount he lost immediately after the crisis. Determinations of the chloride and total base of the serum showed those values to be normal two days before the crisis, and at that time there were also 104 and 241 cc N/10 respectively of those ions in the 24 hour urine. Following the crisis there was a sudden large increase of acid and base radicals, chiefly chloride and sodium, in the urine, the maximum values being 1641 cc N/10 of chloride, and 1791 cc N/10 of base on the second day after the crisis. Coincidentally, the volume of the urine nearly doubled. This patient therefore, although acting in the expected manner with regard to his weight changes, did not show either a low blood chloride and base, or a low urinary excretion of these ions at the height of his fever.

The second patient, A S, was studied for 5 days preceding his crisis and 9 days succeeding it. *He lost weight*, to the extent of  $3\frac{1}{2}$  pounds, during the first 8 days, (i e 5 days *before*, and 3 days *after* the crisis), and although on a nearly zero salt intake this patient excreted 1084 cc N/10 base, and 442 cc N/10 chloride in urine and stools, during the  $6\frac{1}{2}$  days he was on the metabolism frame. In the blood serum three determinations of base and four of chloride showed steadily decreasing values prior to the crisis and a definitely low chloride as late as 36 hours after the crisis. The urinary values for base and chloride decreased rapidly before the crisis and remained exceedingly small for 4 days thereafter, that is to say for over 2 days after the weight had begun to rise. The patient was put on a full milk diet 48 hours after the crisis, but it was not until 3 days later that base and chloride appeared in the urine in moderately large amounts, amounts nevertheless which did not approach the values achieved by the first patient during his phase of maximum post-critical excretion. In table 1 are given figures which show the extent of loss of base and chloride in the first two periods, (i e while on a very low salt intake), and the markedly positive balance of these ions after a salt containing diet was resumed. (It is perhaps noteworthy that base and chloride appear, throughout, to behave very much in

the same way, and it need hardly be mentioned that by far the largest part of the base referred to is sodium )

From the preceding it is evident that the behavior of the 2nd patient was exactly opposite to that of the first To be sure his serum and urinary chloride and base dropped to low levels, but contrary to the

TABLE I  
*Average base and chloride balances per 24 hours in patient A S*

|                            | Period I (36<br>hours) low<br>salt diet | Period II (5<br>days) low<br>salt diet | Period III (6<br>days) high<br>salt diet |
|----------------------------|---|--|--|
| Fixed base cc. N/10        |   |  |  |
| Intake.                    | 0                                       | 0                                      | 1 433                                    |
| Output in urine            | 130                                     | 27                                     | 285 5                                    |
| Output in stools           | 300                                     | 61                                     | 206 5                                    |
| Total output               | <u>430</u>                              | <u>88</u>                              | <u>492 0</u>                             |
| Balance                    | -430                                    | -88                                    | +941                                     |
| Chloride, cc. N/10         |   |  |  |
| Intake                     | 0                                       | 0                                      | 374                                      |
| Output in urine            | 152                                     | 13                                     | 144                                      |
| Output in stools           | 48                                      | 15 5                                   | 17                                       |
| Total output.              | <u>200</u>                              | <u>28 5</u>                            | <u>161</u>                               |
| Balance                    | -200                                    | -28 5                                  | +213                                     |
| Water, cc.                 |   |  |  |
| Intake.                    | 875                                     | 854                                    | 1 284                                    |
| Output (urine and stools)  | <u>509</u>                              | <u>490</u>                             | <u>858</u>                               |
| Difference                 | +366                                    | +364                                   | +426                                     |
| Weight, grams              |   |  |  |
| Average daily gain or loss | +40                                     | -157                                   | +83                                      |

conventional idea this patient lost much weight and showed a large negative balance of both base and chloride previous to the crisis Chart 1 illustrates graphically the contrast between the findings from these two patients The effort on the part of patient A S to restore salt to the body is shown by the early rise of the weight curve through

## EXPERIMENTAL

Patient W H was studied for the 3 days preceding and the 6 days succeeding his crisis. During the first 3 days he gained nearly 2 pounds in weight, which same amount he lost immediately after the crisis. Determinations of the chloride and total base of the serum showed those values to be normal two days before the crisis, and at that time there were also 104 and 241 cc N/10 respectively of those ions in the 24 hour urine. Following the crisis there was a sudden large increase of acid and base radicals, chiefly chloride and sodium, in the urine, the maximum values being 1641 cc N/10 of chloride, and 1791 cc N/10 of base on the second day after the crisis. Coincidentally, the volume of the urine nearly doubled. This patient therefore, although acting in the expected manner with regard to his weight changes, did not show either a low blood chloride and base, or a low urinary excretion of these ions at the height of his fever.

The second patient, A S, was studied for 5 days preceding his crisis and 9 days succeeding it. *He lost weight*, to the extent of 3½ pounds, during the first 8 days, (i e 5 days *before*, and 3 days *after* the crisis), and although on a nearly zero salt intake this patient excreted 1084 cc N/10 base, and 442 cc N/10 chloride in urine and stools, during the 6½ days he was on the metabolism frame. In the blood serum three determinations of base and four of chloride showed steadily decreasing values prior to the crisis and a definitely low chloride as late as 36 hours after the crisis. The urinary values for base and chloride decreased rapidly before the crisis and remained exceedingly small for 4 days thereafter, that is to say for over 2 days after the weight had begun to rise. The patient was put on a full milk diet 48 hours after the crisis, but it was not until 3 days later that base and chloride appeared in the urine in moderately large amounts, amounts nevertheless which did not approach the values achieved by the first patient during his phase of maximum post-critical excretion. In table 1 are given figures which show the extent of loss of base and chloride in the first two periods, (i e while on a very low salt intake), and the markedly positive balance of these ions after a salt containing diet was resumed. (It is perhaps noteworthy that base and chloride appear, throughout, to behave very much in

the same way, and it need hardly be mentioned that by far the largest part of the base referred to is sodium)

From the preceding it is evident that the behavior of the 2nd patient was exactly opposite to that of the first. To be sure his serum and urinary chloride and base dropped to low levels, but contrary to the

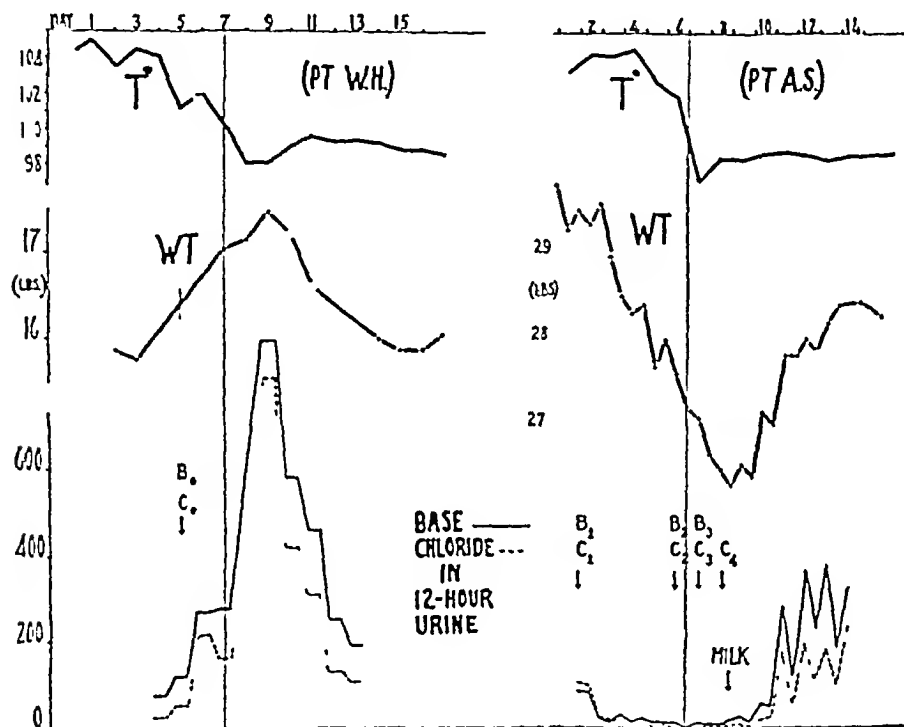
TABLE 1  
*Average base and chloride balances per 24 hours in patient A S*

|                            | Period I (36 hours) low salt diet | Period II (5 days) low salt diet | Period III (6 days) high salt diet |
|----------------------------|-----------------------------------|----------------------------------|------------------------------------|
| Fixed base cc. N/10        |                                   |                                  |                                    |
| Intake                     | 0                                 | 0                                | 1,433                              |
| Output in urine            | 130                               | 27                               | 285 5                              |
| Output in stools.          | 300                               | 61                               | 206 5                              |
| Total output.              | <u>430</u>                        | <u>88</u>                        | <u>492 0</u>                       |
| Balance                    | -430                              | -88                              | +941                               |
| Chloride, cc. N/10         |                                   |                                  |                                    |
| Intake                     | 0                                 | 0                                | 374                                |
| Output in urine            | 152                               | 13                               | 144                                |
| Output in stools           | 48                                | 15 5                             | 17                                 |
| Total output.              | <u>200</u>                        | <u>28 5</u>                      | <u>161</u>                         |
| Balance                    | -200                              | -28 5                            | +213                               |
| Water, cc.                 |                                   |                                  |                                    |
| Intake.                    | 875                               | 854                              | 1,284                              |
| Output (urine and stools)  | <u>509</u>                        | <u>490</u>                       | <u>858</u>                         |
| Difference                 | +366                              | +364                             | +426                               |
| Weight, grams              |                                   |                                  |                                    |
| Average daily gain or loss | +40                               | -157                             | +83                                |

conventional idea this patient lost much weight and showed a large negative balance of both base and chloride previous to the crisis Chart 1 illustrates graphically the contrast between the findings from these two patients. The effort on the part of patient A S to restore salt to the body is shown by the early rise of the weight curve through

immediate retention of salt and water, and the late appearance of salt in the urine when placed on a moderately high salt diet

We soon realized that the key to the difference shown by these two infants lay in the fact that the former received a moderately large amount of NaCl during the course of his illness and retained water,



CHAPT 1 ILLUSTRATING DIFFERENCES IN WEIGHT CURVES AND IN URINARY EXCRETION OF CHLORIDE AND BASE, BETWEEN PATIENT W H ON A MODERATELY HIGH SALT INTAKE AND PATIENT A S ON A VERY LOW SALT INTAKE

Crisis occurred at points marked by vertical lines B<sub>0</sub> = 153, B<sub>1</sub> = 157, B<sub>2</sub> = 149, B<sub>3</sub> = 137 cc N/10 Base in the blood serum, C<sub>0</sub> = 96, C<sub>1</sub> = 96, C<sub>2</sub> = 90, C<sub>3</sub> = 85, and C<sub>4</sub> = 93 cc N/10 Chloride in the blood serum

whereas the latter was given almost no salt and as a result lost weight despite an average daily intake of over 1200 cc of fluid in the form of orange juice and glucose solution

This surmise was abundantly confirmed by a survey of the cases of primary pneumonia in the records of the Infants' Hospital Taking

first a group of infants whose diet had been restricted to orange juice and glucose solution, we found an average salt intake of only 0.7 gram a day or the equivalent in a 20 pound infant of about 6 cc N/10 NaCl per pound body weight. Like patient A S described above, these patients lost much weight previous to the crisis. See chart 2.

In contrast with the above group were other patients which had made gains of 30 to 40 ounces in as short a time as 36 hours. These

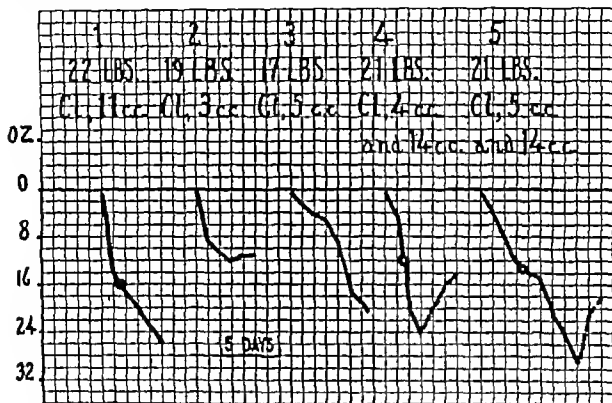


CHART 2 ILLUSTRATING EXTENT OF WEIGHT LOSS OBSERVED IN PATIENTS ON LOW INTAKES OF SALT, EXPRESSED AS CHLORIDE

Dotted line represents weight curve following increase of salt intake. Circle represents crisis.

were all found to have received large amounts of salt in their diets, usually in the form of a mixture of normal saline solution (0.9 per cent), and orange juice. Some of these infants took 6 grams of salt a day, or from 50 to 75 cc. N/10 NaCl per pound body weight, in other words about ten times the amount obtained by the group on a low salt intake. See chart 3.

In addition to the primary differences in the weight curves of infants on low and infants on high salt intakes, charts 2 and 3 illustrate

in certain patients the effect of changing from one diet to another. In chart 2 may be noted two infants, (no 4 and no 5), who lost weight while on salt intakes of 4 and 5 cc N/10 respectively per pound body weight, but who gained rapidly as soon as the intake was increased to 14 cc. The reverse effect is seen in chart 3. Patients no 2, no 4, and no 5 gained in weight when getting 59, 32, and 30 cc N/10 NaCl

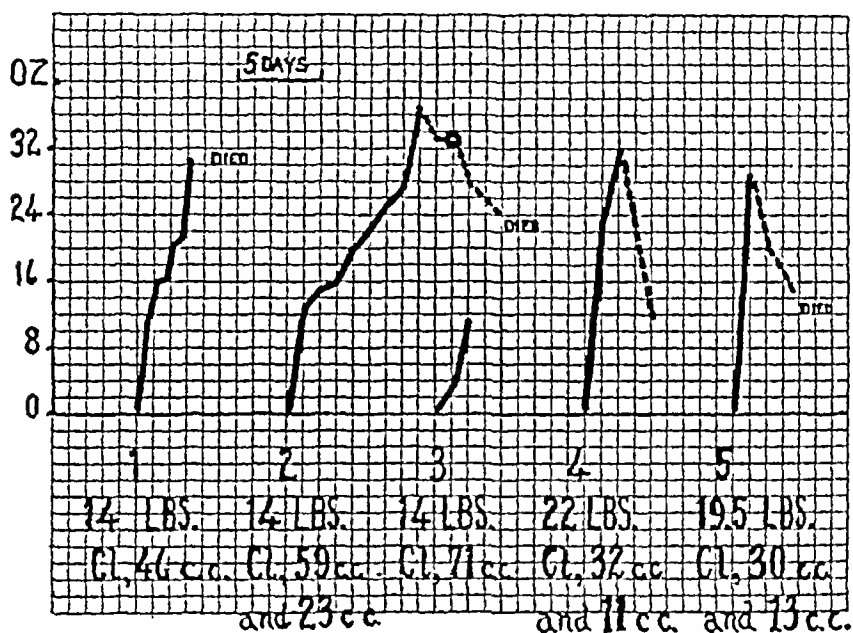


CHART 3 ILLUSTRATING EXTENT OF GAINS IN WEIGHT OBSERVED IN PATIENTS ON HIGH INTAKES OF SALT, EXPRESSED AS CHLORIDE

Dotted line represents weight curve following decrease in salt intake. Circle represents crisis.

per pound body weight, and then lost when the salt was reduced to 23, 11, and 3 cc respectively.

From these findings it seems clear that the patients represented by chart 2 could not retain water until they were also provided with a certain level of salt intake. Like A. S. they lost weight, and like him no doubt they also had a negative base and chloride balance at the time. A negative balance indeed is strongly suggested in these patients by the fact that their weight loss continued beyond the crisis.

until after their salt intakes were increased, indicating that in them there had been no appreciable storage of salt that could be released at the crisis as a sort of endogenous supply. In other words it appears that the hypothesis of chloride retention in pneumonia is incorrect when applied to the group of patients on low chloride diets that show low blood and urine values of base and chloride and are losing weight. Extensive retention of Na and Cl, however *does* occur on a high salt diet but in this event there will be found a normal or high level of base and chloride in the serum, with considerable amounts of these ions entering the urine, chemical findings again, which are contrary to current theories.

A third metabolic study, (included as case no 1 in chart 3), may be mentioned briefly here as a patient that received, under our observation, a relatively large amount of salt in his diet, (i.e. 46 cc N/10 NaCl per pound body weight), and furnished us with some significant post-mortem data. Details of the acid base metabolism and of tissue analyses will form the subject of a succeeding paper, but it is of interest to note here the principal findings, namely, normal serum chloride and base, high urinary chloride and base, rapidly increasing weight with pitting edema, a positive balance of Na and Cl ions, great retention of water and salt in the form of edema fluid, and a marked "retention sec," in the body tissues in general. As the patient was a Mongolian idiot with a congenital lesion of the heart, death was perhaps due to that condition. The grave potential dangers, nevertheless, of high salt therapy are suggested by the intense edema that developed in this infant, a finding that was also recorded in the autopsy reports on patients no 3 and no 5 of chart 3. Although not conclusive evidence against salt therapy it is at least suggestive that among 19 cases of primary pneumonia in infants on whom we could get adequate data, the three deaths that occurred were confined to the small group of five cases that received large amounts of salt in their diet, and as noted above, developed extensive edema of their tissues.

There is little evidence to suggest that edema in these patients might have been due to disease of heart or kidneys, either functional or organic. Indeed, marked efficiency of these organs in the patient mentioned above is indicated by the fact that the concentrating power of the kidneys was high throughout the illness. Parallel with the

increasing elimination of salt, both the concentration and the actual amounts of chloride and fixed base in the urine mounted steeply without showing signs of having approached a maximum prior to death

Why the tissues of the body are so markedly at the mercy of the salt intake in pneumonia, despite normally functioning kidneys is not clear. A hint of a specific effect referable to the pneumococcus is offered by the fact that intense general edema sometimes develops in the presence of a pneumococcus abscess, only to vanish when the abscess is drained. On the other hand it is possible that other febrile diseases do manifest in lesser degree the same sort of phenomena, described above, that in pneumonia are rendered especially striking because of the contrasts this disease furnishes in its abrupt onset and its termination by crisis

#### DISCUSSION

The data given in this paper deal principally with the relation in pneumonia of salt intake to salt and water retention, and appear to show that the latter depends largely on the former

Leiter (14) has already pointed out that any study of salt metabolism must take into consideration the salt intake for several days previous to the study. This generalization we believe applies to primary pneumonias, and the reason so many cases described in the literature have a low blood chloride when first seen may well be that these patients correspond with our infants on low salt intakes by virtue of having refused or neglected to take salt-containing foods during the prodromal stage of the illness, previous to admission to the hospital. Findings in this type of case have come to be regarded as characteristic of the disease only because the majority of pneumonia patients desire water and fruit juices (or are offered nothing else), and so become established on a nearly salt-free diet

The opposite situation of high salt intake reveals in pneumonia an obligatory retention of salt and water which is most striking. In extreme cases the edema becomes so great as to be possibly dangerous. It is also conceivable that the incidence of effusions into serous cavities is greater in patients on high than in patients on low salt diets. Consequently it has become our practice to observe caution with salt in pneumonia, giving enough however to keep the patient from losing

weight, namely, about 15 cc. N/10 NaCl per pound body weight, per 24 hours. This quantity is supplied by a solution made up of 1 part saline (0.9 per cent), 2 parts orange juice, and three parts 10 per cent glucose, provided the patient takes 2 ounces of the mixture per pound body weight in 24 hours, thus far cases of pneumonia so treated have maintained nearly stationary weights, and have not developed edema.

### CONCLUSIONS

1 In infants with primary pneumonia we find a negative base and chloride balance if the intake of these ions is low, and a markedly positive balance if the intake is high.

A. In the former situation one observes low values for total base and chloride in the serum, rapidly diminishing values for these ions in the urine, rapid loss of weight, and later a gain in weight only after the salt intake has been adequately increased.

B. In the latter situation the serum values are at least normal, the base and chloride of the urine are normal or high, weight rises rapidly before the crisis, (often with visible edema), and after the crisis there is usually a sharp loss in weight accompanied by an outpouring of chloride and base in the urine.

2 An extensive and perhaps a dangerous degree of edema is often seen to develop in patients who receive large quantities of salt during the early stages of their pneumonia. These patients, despite normal heart and kidneys, appear to be forced to retain in their tissues amounts of salt which a normal individual would excrete without difficulty.

3 In an attempt to maintain an even weight in patients with pneumonia, we have administered 15 cc. N/10 salt solution per pound body weight per twenty-four hours, with satisfactory results.

### BIBLIOGRAPHY

- 1 Peabody, F. W., *J. Exper. Med.*, 1912, xvi, 701. The Carbon Dioxide Content of the Blood in Pneumonia.
- 2 Peabody, F. W., *J. Exper. Med.*, 1913, xvii, 71. Studies of the Inorganic Metabolism in Pneumonia with Especial Reference to Calcium and Magnesium.
- 3 McLean, F. C., *J. Exper. Med.*, 1915, xxii, 366. The Numerical Laws Governing the Rate of Excretion of Urea and Chlorides in Man. II. The Influence of Pathological Conditions and of Drugs on Excretion.

- 4 Maver, Mary E , and Schwartz, A B , Arch Int Med , 1916, xii, 459  
Studies of Edema in Pneumonia
- 5 Lusk, H O , and Friedstein, H , Am J Dis Child , 1920, xix, 337 Water  
Retention in Pneumonia
- 6 Clausen, S W , Arch Int Med , 1925, xxxv, 571 The Excretion of Organic  
Acids after Pneumonia
- 7 Haden, R L , J Lab and Clin Med , 1925, v, 337 The Relation of the  
Chloride Metabolism to the Toxemia of Lobar Pneumonia (Preliminary  
Report )
- 8 Haden, R L , Am J Med Sci , 1927, clxxiv, 744 The Clinical Significance  
of the Chloride Metabolism in Lobar Pneumonia
- 9 Soule, H C , Buckman, T E , and Darrow, D C , J Clin Invest , 1928, v,  
229 Blood Volume in Fever
- 10 Peters, J P , Bulger, H A , Eisenman, A J , and Lee, C , J Biol Chem , 1926,  
lxvii, 219 Total Acid Base Equilibrium of Plasma in Health and Disease  
V Miscellaneous Pathologic Conditions
- 11 McIntosh, J F and Reimann, H A , J Clin Invest , 1926, iii, 123 Kidney  
Function in Pneumonia
- 12 Sunderman, F W , Austin, J H , and Camac, J G , J Clin Invest , 1926,  
iii, 37 Studies in Serum Electrolytes I Concentration of Electrolytes  
and Non-electrolytes in the Serum during Lobar Pneumonia
- 13 Gerstenberger, H J , Burhans, C W , Smith, D N and Wetzel, N C , Am  
J Dis Child , 1923, xxvi, 329 The Blood Serum Content of Inorganic  
Phosphorus and Calcium in Pneumonia
- 14 Leiter, L , J Clin Invest , 1927, iii, 253 Influence of Previous Salt Regime  
on Excretions of Chlorine, Sodium and Potassium during the Chloride  
Concentration Test of De Wesselow

# AN INVESTIGATION OF VARIOUS FACTORS WHICH AFFECT THE SEDIMENTATION RATE OF THE RED BLOOD CELLS<sup>1</sup>

By M. DOROTHY ROURKE AND E. D. PLASS

(From the Beth Israel Hospital, Boston, and the Department of Obstetrics and Gynecology, State University of Iowa)

(Received for publication March 26 1929)

Since the work of Fahraeus, in 1921 (1), the phenomenon of the settling of the red corpuscles in blood to which an anticoagulant has been added has been studied intensively by biologists as well as by clinicians. The former have been interested chiefly in determining the mechanism whereby the settling rate of the cells is increased or diminished in certain physiological and pathological conditions, and in studying the various chemical and physical factors which may vary the rate, while the clinical investigators have attempted to evaluate the diagnostic and prognostic significance of observed changes in the rate of sedimentation. Preliminary to a renewed effort along clinical lines, we have carried out a series of experiments designed to show the effect which certain factors may have upon the settling rate.

The sedimentation tubes used in these experiments contained 10 cc. of blood, were 40 mm. in diameter, were sealed flat at one end, and were graduated from 0 to 80 mm. They were coated on the inside with a very thin layer of paraffin. All blood samples were collected without stasis and were manipulated entirely under oil.<sup>2</sup>

## THE EFFECT OF VARIOUS ANTICOAGULANTS UPON THE RATE OF SEDIMENTATION

The precipitation of colloids of the suspensoid type by adsorption of an electrolyte and the stabilizing of an otherwise labile system by

<sup>1</sup> A portion of the experimental work was completed in 1926 at the Henry Ford Hospital, Detroit.

<sup>2</sup> These tubes were 20 mm. shorter than those finally decided upon and described for clinical use in the *J. Clin. Invest.*, 1928, v, 531.

a similar process are familiar phenomena. Fahraeus (1) noted that, if defibrinated horse blood is diluted with a 0.9 per cent solution of sodium chloride, the specific gravity and viscosity of which are less than that of the serum, the sedimentation rate of the erythrocytes is actually diminished. Furthermore, he showed that if as little as 0.025 gram of solid sodium chloride is added to 5.0 cc of defibrinated horse blood, the sinking of the cells is decreased from 75 to 3 mm in 15 minutes, and that there occurs an increase in the negative charge on the corpuscles, proportional to the amount of sodium chloride added. In these instances, the suspension has been stabilized by addition of the electrolyte. Popper and Kreindler (2) have likewise shown the stabilizing effect of the addition of electrolytes to the blood. On the other hand, Linzenmeier (3) and Fahraeus (1) have both shown that the sedimentation rate increases if small amounts of lanthanum nitrate are added, and that when larger amounts of this salt are introduced the corpuscles of human blood become positively charged. Provided the magnitude of this charge is sufficiently large the system can be made stable. The precipitating effect of some salts and the stabilizing effect of others is probably referable to the valency of the cation, as pointed out by Oliver and Barnard (4).

Because of the changes that added electrolytes may bring about in the stability of the blood, it seemed advisable to determine the effect of the different anticoagulants in common use, and to adopt for clinical work a substance which has no effect upon the suspension stability other than keeping the blood from clotting. Sodium fluoride (50 mgm per 5.0 cc of blood), potassium oxalate (14 mgm per 5.0 cc of blood), and potassium citrate (10 mgm per 5.0 cc of blood) were taken as representative of the inorganic anticoagulants, and were compared with each other and with heparin (1 mgm per 5.0 cc of blood), the organic anticoagulant obtained from liver substance. In every instance the concentration of anticoagulant was sufficient to prevent clotting during the period of the experiment.

Chart 1 presents graphically the varied rates of settling found when separate specimens of the same blood were treated with the different anticoagulants in the amounts indicated above. With the settling most rapid in the heparinated sample, it is apparent that the rate of sedimentation in the others varies inversely with the concen-

tration of salt necessary to prevent coagulation. Similar differences were noted in several other experiments of the same nature. No attempt was made to determine whether the slowing varies mathematically with the concentration of the salts.

In the Linzenmeier (3) method employed by a great many investigators for the clinical determination of the sedimentation rate, one part of 5 per cent sodium citrate is used to four parts of blood. Such

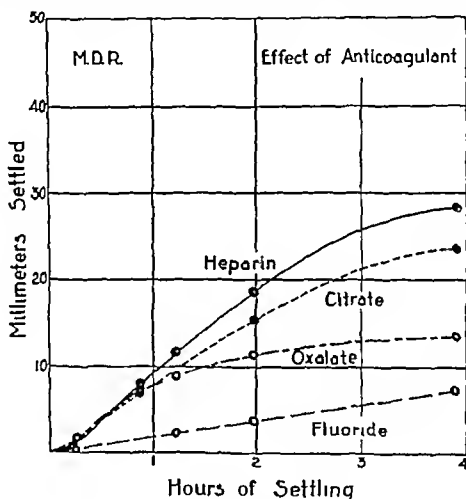


CHART 1 THE EFFECT OF DIFFERENT ANTICOAGULANTS UPON THE SEDIMENTATION RATE OF BLOOD CELLS

a mixture was compared with blood treated with dry potassium citrate (10 mgm per 5 cc of blood) and with heparin (1 mgm. per 5 cc of blood). The results are shown in chart 2. The diluted blood (the curve) settled more slowly even than the blood treated with dry citrate. In the diluted specimen, the concentration of citrate was equal to 50 mgm. in 5 cc. of blood, a fact which undoubtedly explains the slower settling. The dilution itself would increase

the rate, so that the real effect of the increased salt concentration is actually much greater than is apparent

It now became essential to learn whether heparin has some active influence toward hastening the settling, or whether the results obtained when this substance is used represent the sedimentation rate of untreated blood. It seemed reasonable to assume that if a certain concentration of any anticoagulant affects the stability of the blood,

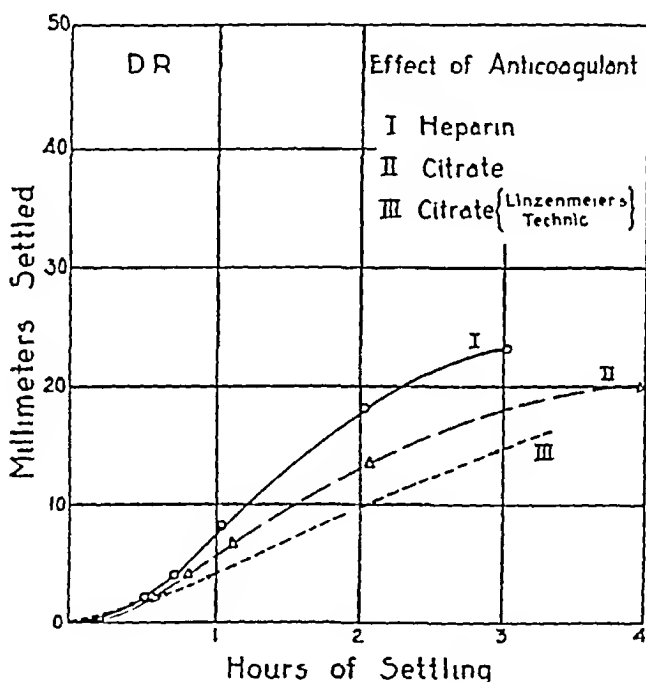


CHART 2 THE EFFECT UPON THE SEDIMENTATION RATE OF SOLID CITRATE AND CITRATE SOLUTION AS COMPARED WITH THE EFFECT OF HEPARIN

a greater concentration should produce a more marked effect in the same direction. Doubling the concentration of the oxalate, the citrate, or the fluoride always produces an appreciably greater slowing of the sedimentation rate, whereas, even when the concentration of heparin is tripled or quadrupled there is no effect upon the rate of settling (chart 3).

It is obvious that the concentration in the plasma of the anticoagulant will be less in those bloods with high plasma concen-

percentages than in those with higher hematocrits. For this reason, the stabilizing effect of the electrolyte anticoagulants varies from one blood to another, and the results are not strictly comparable. Heparin should then be the preferred anticoagulant for this type of

### Rate Of Sedimentation Of Red Blood Cells

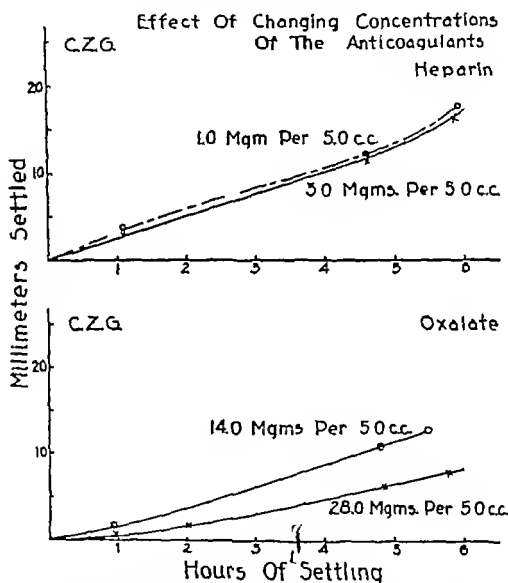


CHART 3 THE EFFECT UPON THE SEDIMENTATION RATE OF INCREASING THE CONCENTRATION OF DIFFERENT ANTICOAGULANTS (OXALATE AND HEPARIN)

work, for this substance even in considerable concentration does not affect the sedimentation rate. Heparin was, therefore, used as the anticoagulant in all the experiments reported below. Absolute proof that heparin does not affect the sedimentation rate was not easy, but finally we were fortunate enough to secure a

sample of blood from a hemophiliac, whose clotting time was over five hours Chart 4 shows the sedimentation curves in two specimens of this blood, the one with no anticoagulant and the other with heparin (1 mgm per 5 cc of blood) Since there was no demonstrable difference in the settling rates of the two samples, we were forced to the conclusion that heparinated blood settles more rapidly than blood treated with fluoride, citrate, or oxalate not because heparin accelerates

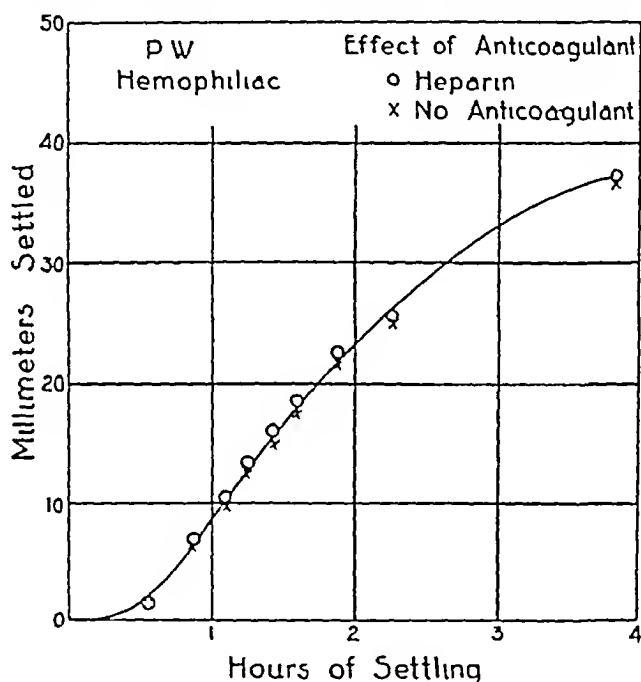


CHART 4 NO EFFECT UPON THE SEDIMENTATION RATE OF HEMOPHILIAC BLOOD WHEN HEPARIN IS ADDED

settling, but solely because it has no appreciable effect, whereas the electrolyte anticoagulants produce a definite slowing

#### CHANGES IN THE STABILITY OF THE BLOOD AFTER STANDING AT ROOM TEMPERATURES

Pohle (5) states that if blood is allowed to stand and is then re-shaken, the sedimentation velocity is rather markedly accelerated as compared with freshly drawn blood Westergren (6), however,

found that preserving blood for several hours at room temperature does not materially affect its settling velocity, but that after twenty-four hours standing the rate is slowed. Fahraeus (1), working with defibrinated horse blood reports that a specimen may be allowed to stand at room temperature for as long as seven hours without changing

TABLE 1  
*Heparin—our technic*

| Freshly drawn | Millimeters settled in one hour after standing |         |         |         |         |         |         |         |
|---------------|--|---------|---------|---------|---------|---------|---------|---------|
|               | 1 hour   | 2 hours | 3 hours | 4 hours | 5 hours | 6 hours | 7 hours | 8 hours |
| 42            |  | 41      |         | 37      |         | 36      |         | 33      |
| 44            | 44   | 43      | 44      |         |         |         |         |         |
| 40            |  | 40      | 39      | 40      | 40      |         |         |         |
| 39            |  | 39      | 39      | 39      | 38      |         |         |         |
| 35            |  | 35      | 33      | 35      |         |         |         |         |
| 48            |  | 46      | 46      | 46      | 46      |         |         |         |
| 19            |  | 20      |         | 21      |         | 21      |         | 22      |
| 8             |  | 7       |         | 8       |         | 8       |         | 13      |
| 6             |  | 5       |         | 7       |         | 6       |         | 6       |
| 5             |  | 5       |         | 5       |         | 6       |         | 6       |

TABLE 2  
*Sodium citrate solution—Lanzetta's technic*

| Freshly drawn | Millimeters settled in one hour after standing |         |         |         |         |         |         |          |          |
|---------------|--|---------|---------|---------|---------|---------|---------|----------|----------|
|               | 1 hour   | 2 hours | 3 hours | 4 hours | 5 hours | 6 hours | 7 hours | 12 hours | 24 hours |
| 15            |  |         | 12      |         | 12      |         | 13      | 12       |          |
| 11            |  |         | 11      |         | 10      |         | 10      | 10       |          |
| 12            |  |         | 10      |         | 9       |         | 10      | 8        |          |
| 4             |  |         | 4       |         | 3       |         | 4       | 4        |          |
| 35            |  | 35      |         | 34      |         | 35      |         |          | 21       |
| 53            |  | 53      |         | 53      |         | 54      |         |          | 44       |
| 46            |  | 45      |         | 44      |         | 44      |         |          | 38       |
| 48            |  | 49      |         | 49      |         | 49      |         |          | 48       |

the amount of rouleau formation or the sedimentation velocity, but that when such blood is kept at higher temperatures (up to 58°C) rouleau formation is diminished, with a minimum at 42°C. When he allowed a sample of oxalated blood to stand for 5 hours at 28°C and another similar sample at 18°C for the same period, the former

settled more slowly Wiltshire (7) has shown that oxalated human plasma loses more and more of its aggregating power on standing at room temperature Obviously, it is of importance to learn definitely how much time may elapse between the collection of blood and the completion of the sedimentation test without any sacrifice of accuracy

The following experiments were performed to measure changes in stability on standing with our method of preventing coagulation with heparin, as well as with Linzenmeier's method using one volume of 5 per cent sodium citrate to four volumes of blood The bloods stood at temperatures of 22° to 25°C and settling took place at the same temperatures

[Blood tends to become more stable on standing, but within the period of a working day the changes are usually insignificant, while after twenty-four hours at room temperature a considerable slowing of the sedimentation rate is usually observed The effects of heparin and sodium citrate solution are practically the same)

Such results lead us to conclude that, for clinical purposes, blood sedimentation determinations may be postponed for some hours (6 to 12) after the blood is drawn without any considerable loss of accuracy, but that standing for a longer period may lead to serious errors by slowing the settling rate

We are at a loss to explain why our results and Westergren's do not agree with those of Pohle, for we have found the reaction completely reversible within the time limits noted Our blood samples were manipulated entirely under oil, but since Westergren does not state that he took a similar precaution, it seems doubtful whether that factor can explain the varied results

#### THE EFFECT ON THE RATE OF SEDIMENTATION OF CENTRIFUGING AND REMIXING THE CELLS AND PLASMA

It was necessary in some of our experimental work to centrifuge the blood and later remix cells and plasma for a second determination of the sedimentation rate To determine whether such manipulations have any effect upon the settling velocity, several specimens were treated in this way and the sedimentation rates compared with those of the original samples

*Experiment 1* E L M, normal young woman A specimen was

set up at once for determination of the sedimentation rate and the remainder immediately centrifuged for 20 minutes at 2500 r p m. This latter specimen was then remixed and the rate measured. The settling curves for the two specimens are identical (See chart 5)

*Experiment 2* Dog A. Distemper. The blood was treated as in experiment 1 and the settling curves are identical (chart 5). It is

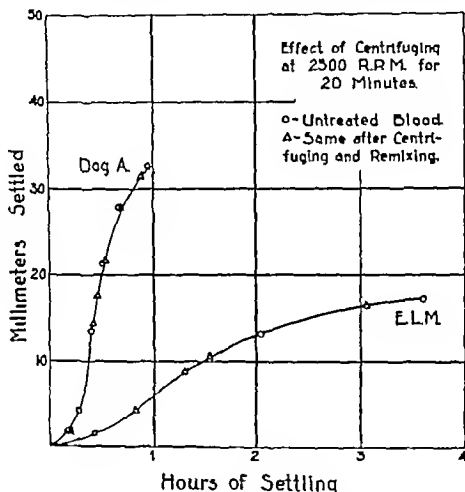


CHART 5 NO EFFECT UPON THE SEDIMENTATION RATE AFTER CENTRIFUGING BLOOD FOR 20 MINUTES AT 2500 R.P.M. AND REMIXING

important to note that rapidly-settling bloods are as reversible after centrifuging as are normal bloods

*Experiment 3* M D R, normal young woman. The blood was treated as in experiment 1, except that after centrifuging the second sample and before remixing, the blood was allowed to stand for two hours at room temperature. The untreated specimen settled 14 mm in one hour, while the other settled 15 mm—an insignificant difference.

We have done other experiments of this character as well as a

limits In a typical experiment reducing the carbon dioxide content from 69 to 7 per cent increased the sedimentation rate from 20 to 22 mm in one hour, while increasing the oxygen content from 6 to 16 per cent increased the settling from 9 to 11 mm in one hour Although these changes are very small they are consistent with several other

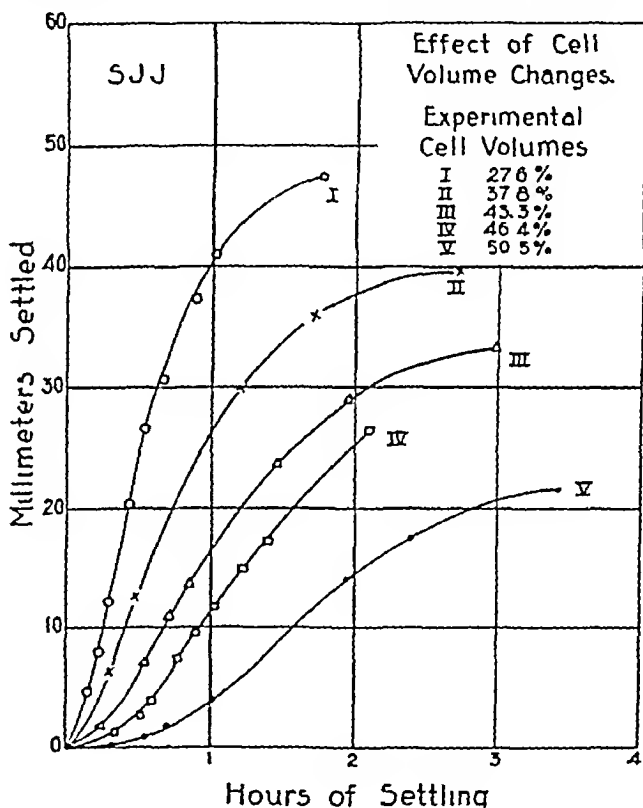


CHART 7 THE EFFECT UPON THE SEDIMENTATION RATE OF A SLOWLY SETTLING BLOOD PRODUCED BY CHANGING THE CELL VOLUME

experiments recorded by this author It would seem that simple aeration should produce only insignificant variations

Our experimental procedure was as follows Four cubic centimeters of blood were drawn from an arm vein under oil, using heparin as the anticoagulant, and the sedimentation rate was determined as

and agitated by rotating until the blood had become bright scarlet in color, after which its sedimentation rate was determined by the usual procedure. The time of reading was varied so that packing was not a factor.

The results of this study are in accord with what might be expected from the experiments of Popper and Kreindler (2), and of Ito (15)

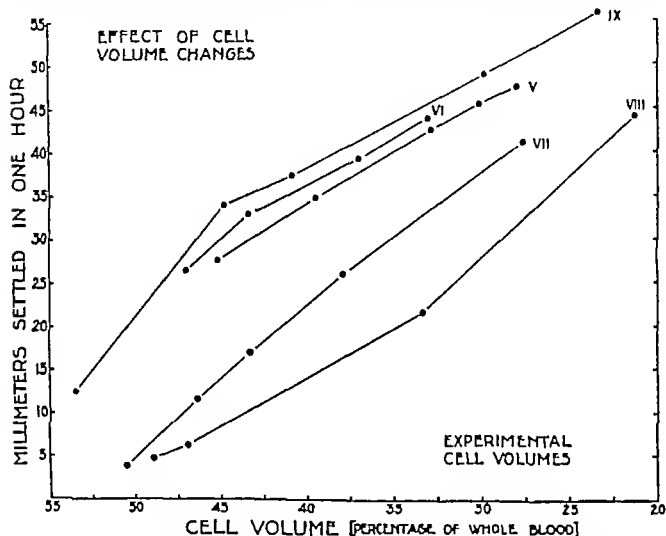


CHART 8 THE MILLIMETERS SETTLED IN ONE HOUR PLOTTED AGAINST THE CELL VOLUME

Five experiments in which the cell volume was artificially regulated

We conclude that the sedimentation rate is not appreciably affected by aeration or by loss of carbon dioxide, and that for practical clinical purposes collection under oil is not necessary, although this precaution would seem indicated where unusual accuracy is desirable. This conclusion would seem applicable to both slowly and rapidly settling bloods.

## THE EFFECT OF TEMPERATURE UPON THE SEDIMENTATION RATE

DeCourcy (8), using the Linzenmeier technic, determined the sedimentation rates of various blood samples at 6°, 18°, and 37°C and concluded that while there was only a slightly increased rapidity of settling between 6° and 18°C, there was a considerable increase between 18° and 37°C. Westergren (6) shows graphically the variations in sedimentation rates at different temperatures, 14° to 25°C, of bloods settling with various speeds, and suggests the use of similar curves for correctional purposes. He also found that the effect of an increase in temperature is smaller with slowly settling

TABLE 3  
*The effect of aeration of the blood upon the sedimentation rate*

| Blood number | Time settled | Millimeters settled |                |
|--------------|--------------|---------------------|----------------|
|              |              | Sample as drawn     | Aerated sample |
| 1            | 1 hour       | 10                  | 10             |
| 2            | 1 hour       | 11                  | 14             |
| 3            | 1 hour       | 12                  | 12             |
| 4            | 1 hour       | 6                   | 4              |
| 5            | 1 hour       | 8                   | 9              |
| 6            | 1 hour       | 3                   | 3              |
| 7            | 1 hour       | 9                   | 8              |
| 8            | 20 minutes   | 32                  | 32             |
| 9            | 20 minutes   | 28                  | 31             |
| 10           | 10 minutes   | 33                  | 33             |

bloods and that the effect of a definite (e.g., a 5°C) variation in temperature is greater as the temperature increases. Gordon and Cohn (16), using Linzenmeier's method, studied the sedimentation rates at 10°, 23°, and 37°C and found startling differences in all the rates between these temperatures. They also found that the effect of external temperatures within these limits is transitory, i.e. that upon shaking and retesting the blood at a second temperature no influence of the original temperature will be apparent. These authors conclude that since the average room temperature varies between 22° and 24°C, a standard of 23°C should be maintained during the determination of the settling rate.

We have determined the sedimentation by our method<sup>3</sup> on specimens of the same samples of blood at different temperatures varying from 20° to 38°C, and our findings in the main are similar to those noted above. Carefully regulated water baths were used for all the determinations, and the samples were allowed to remain in the baths for 30 minutes before the tests were started in order to obtain temperature equilibrium. Chart 9 shows graphically the curves obtained in

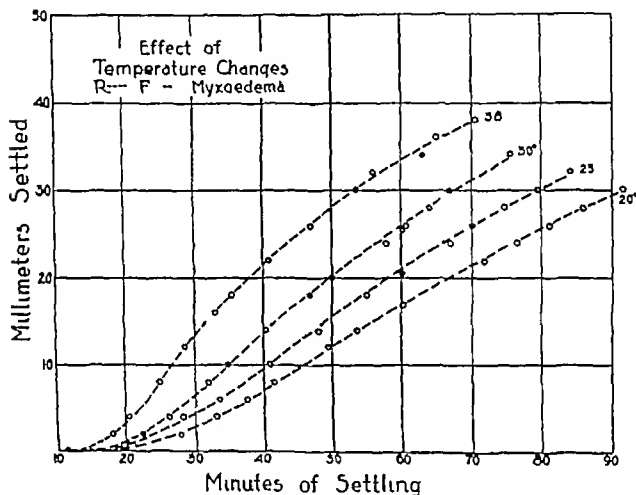


CHART 9 THE EFFECT OF TEMPERATURE CHANGES UPON THE SEDIMENTATION RATE

a typical experiment where the blood was maintained at four different temperatures ranging from 20° to 38°C. The findings in 13 other experiments are presented in table 4.

It is obvious that the settling rate increases to an appreciable extent with rising temperature within the range with which we are dealing, and that the increase in rate is less from 20° to 25° than from 25° to

<sup>3</sup> Tubes giving a column of blood 100 mm. high have been used for these temperature experiments. (See J Clin Invest., 1928, v, 531.)

30°C Furthermore, the relative increase of rate per degree of temperature from 30° to 38° is greater than from 20° to 25° in the slowly settling specimens

In more rapidly settling bloods, however, packing becomes such an important factor in the one hour readings at 38° that the true temperature effect is masked and the curve falls off In specimen 13, infectious arthritis, packing has almost entirely masked the temperature change

TABLE 4  
*The effect of temperature changes on the sedimentation rate*

| Subject  | Sex | Diagnosis                | Millimeters settled in one hour at |     |     |     |
|----------|-----|--------------------------|------------------------------------|-----|-----|-----|
|          |     |                          | 20°                                | 25° | 30° | 38° |
| I C W M  | M   | Normal                   |                                    |     | 2   | 3   |
| II W M   | F   | Normal                   | 2                                  | 2.5 | 4   | 6   |
| III M R. | M   | 2nd and 3rd degree burns |                                    |     | 6   | 11  |
| IV D G   | F   | Normal                   | 6                                  | 7   | 10  | 14  |
| V Dog B  | F   | Normal                   |                                    |     | 9   | 14  |
| VI B J   | M   | Normal                   | 9                                  | 11  | 15  | 20  |
| VII R R  | M   | Normal                   | 12                                 |     | 19  | 23  |
| VIII R F | F   | Myxedema                 | 17                                 | 20  | 26  | 34  |
| IX B M   | M   | Tuberculosis (mild)      | 23                                 | 26  | 30  | 33  |
| X L G    | M   | Rheumatic fever          | 28                                 | 32  | 39  | 40  |
| XI H H   | M   | Arsphenamine dermatitis  | 37                                 | 39  | 46  | 48  |
| XII R F  | M   | Tuberculosis             | 40                                 |     | 46  | 48  |
| XIII A R | M   | Infectious arthritis     | 59                                 | 59  | 59  | 60  |

We can not suggest the use of curves similar to those presented in chart 9 for correctional purposes, since the type of curve for rapidly settling bloods is dependent upon the relative importance of the packing factor, which would produce a greater straightening of the temperature rate curve for bloods with higher hematocrits (greater cell volumes)

For clinical work, we suggest that all tests be carried out at a room temperature of 20° to 25°C, since this range is not difficult to maintain in most laboratories, and since we feel that the variations in rate at such temperatures are not great enough to affect the value of

the results Any apparatus, which might be used to control the temperature more closely (constant temperature baths or rooms) would not be practical for clinical work, and so can not be advocated. However, if tests must be made under unusual temperature conditions, such as extreme summer heat, an attempt should be made to control the temperature between  $20^{\circ}$  and  $25^{\circ}\text{C}$ , or due allowance must be made in interpreting results. It is obvious that it is only in middle range (doubtful cases) that the effect of temperature changes might interfere with the interpretation of results.

#### THE EFFECT OF INGESTION OF FOOD UPON THE STABILITY OF THE BLOOD

Sedimentation rates and plasma protein fractions (Wu's (17) method) were determined upon blood samples from two subjects immediately before and shortly after the ingestion of food. In another experiment (no 3), the sedimentation rate was determined at 11 00 a.m. and at 4 00 p.m., the subject not having eaten since 8 00 a.m. In the last experiment (no 4) blood samples were taken for the determination of settling rates four hours after breakfast and again four hours after lunch on the same day.

The changes noted are quite insignificant and confirm DeCourcy's (7) observations. It seems doubtful whether there is any reason to prescribe that tests for clinical purposes should be run at any particular time of the day.

#### THE EFFECT OF SHORT, VIOLENT EXERCISE UPON THE SEDIMENTATION RATE

The effect of muscular exercise in concentrating the blood (increased plasma protein percentage and decreased plasma volume percentage) has been thoroughly studied by Scott, Hermann, and Snell (18), Peters, Bulger, Eisenmann, and Lee (19), and Rowe (20).

In view of this concentration, it would be reasonable to expect that there would be some alteration of the settling rate of the blood cells, but we can find no record of an experimental investigation of this point. Our experiments were made upon healthy, young individuals, blood samples being collected just before and immediately after the exercise period. Plasma proteins were determined by the method of Wu (17), the plasma carbon dioxide by the Van Slyke and Stadie

TABLE 5

*Effect of ingestion of food upon stability of the blood*

Experiment 1 E S, normal young woman

| Specimen                                | Plasma          |                 |                 |                 | Cell volume     | Settling after 1 hour |
|---|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------------|
|   | Fibrin          | Globulin        | Albumin         | Total protein   |                 |                       |
|   | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |                       |
| No 1—11 30 a m, 4 hours after breakfast | 0 34            | 2 66            | 4 64            | 7 64            | 44 9            | 13                    |
| No 2—1 30 p m, 1 hour after lunch       | 0 31            | 2 62            | 4 54            | 7 47            | 42 9            | 16                    |

Experiment 2 C W M, normal young man

| Specimen                                 | Plasma          |                 |                 |                 | Cell volume     | Settling after 1 hour |
|--|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------------|
|  | Fibrin          | Globulin        | Albumin         | Total protein   |                 |                       |
|  | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |                       |
| No 1—11 30 a m, 3½ hours after breakfast | 0 32            | 2 42            | 4 95            | 7 69            | 46 9            | 3                     |
| No 2—1 30 p m, 1 hour after lunch        | 0 30            | 2 60            | 4 93            | 7 83            | 45 0            | 3                     |

Experiment 3 M D R, normal young woman

| Specimen   | Cell volume     | Settling after 1 hour |
|--|-----------------|-----------------------|
|  | <i>per cent</i> |                       |
| No 1—11 00 a.m, 3 hours after breakfast          | 42 7            | 14                    |
| No 2—1 00 p m, no lunch, 8 hours after breakfast | 43 2            | 17                    |

Experiment 4 J L M, normal young woman

| Specimen                                | Cell volume     | Millimeters settled in 1 hour |
|---|-----------------|-------------------------------|
|   | <i>per cent</i> |                               |
| No 1—11 30 a m, 4 hours after breakfast | 43 6            | 6                             |
| No 2—1 30 p m, 4 hours after lunch      | 43 6            | 6                             |

procedure (21), and the sedimentation rate and cell volume on heparinated blood according to our own technic.

Short, violent exercise of this nature tends to concentrate the blood,

increasing the plasma protein percentage, which should, according to our concept, make for more rapid settling of the cells, and at the same time increasing the cell volume percentage, which should retard sedimentation. The observed decrease in the carbon dioxide content of the plasma should have no appreciable effect on the sedimentation

TABLE 6

*Effect of exercise upon the sedimentation rate*

Experiment 1 R. L. J., a healthy young man ran up and down four flights of stairs four times, taking three minutes for the task, and being quite exhausted at the end

|                 | Plasma   |          |          |               | CO <sub>2</sub> content | Heparin cell volume | Settled in 1 hour |
|-----------------|----------|----------|----------|---------------|-------------------------|---------------------|-------------------|
|                 | Fibrin   | Globulin | Albumin  | Total protein |                         |                     |                   |
|                 | per cent | per cent | per cent | per cent      | volumes per cent        |                     |                   |
| Before exercise | 0.25     | 2.10     | 4.80     | 7.15          | 49.0                    | 45.5                | 6                 |
| After exercise  | 0.27     | 2.32     | 4.95     | 7.54          | 38.0                    | 48.0                | 8                 |

Experiment 2 M. D. R., a healthy young woman, ran up and down three flights of stairs six times in four minutes

|                 | Plasma   |          |          |               | Heparin cell volume | Settled in 1 hour |
|-----------------|----------|----------|----------|---------------|---------------------|-------------------|
|                 | Fibrin   | Globulin | Albumin  | Total protein |                     |                   |
|                 | per cent | per cent | per cent | per cent      |                     |                   |
| Before exercise | 0.31     | 2.12     | 4.40     | 6.85          | 45.6                | 7                 |
| After exercise  | 0.35     | 2.22     | 4.68     | 7.25          | 48.1                | 6                 |

Experiment 3 P. M. H., a healthy young woman ran up and down three flights of stairs three times in two minutes

|                 | Heparin cell volume | Settled in 1 hour |
|-----------------|---------------------|-------------------|
| Before exercise | 40.4                | 11                |
| After exercise. | 44.2                | 11                |

velocity, according to the studies of Ito (15). Assuming that other factors, such as increased lactic acid, have relatively negligible effects, it would seem that, in the experiments noted, the increase in plasma proteins, particularly the fibrin fraction, produces an accelerating effect, which is approximately (in experiment 3 exactly) equalized

by the retarding effect of the increase in cell volume percentage. The subject warrants more study, but in the light of these experiments, we feel that the sedimentation velocity of the blood cells could not be altered materially by any usual state of activity of the individual.

#### CONCLUSIONS

1 Heparin is the ideal anticoagulant for blood sedimentation studies, since it has no effect on the settling rate other than keeping the blood from clotting. The inorganic anticoagulants depress the rate of settling in proportion to their concentration. Dilution with the citrate solution described in Luzenmeyer's technic slows the rate to a greater extent than does the dry salt.

2 Aggregation of the red blood cells under conditions obtaining in sedimentation tests is a completely reversible phenomenon. Blood may usually stand at room temperature for six to twelve hours without the sedimentation rate being affected materially, whereas longer standing generally leads to a slowing of the rate. All tests should be completed on the day when the blood is drawn.

3 Centrifuging blood for 20 minutes at 2500 r p m does not affect its settling velocity after removing.

4 Blood dilution (with its own plasma) leads to more rapid settling of the cells. In samples where the aggregating and packing factors are not largely operative this change is represented by a straight-line curve.

5 Aeration of venous blood has no significant effect upon the rate of settling, therefore the collection of blood samples under oil is not necessary.

6 Increase of temperature (within certain limits) makes for more rapid sedimentation. Within the range of ordinary room temperatures, the changes are hardly significant.

7 The changes in rate of settling due to the ingestion of food are slight and may be disregarded for ordinary work.

8 Short, violent exercise has an insignificant and variable effect on the sedimentation rate. The accelerating effect of the fibrinogen increase is compensated by the retarding effect of the increase in cell volume.

## BIBLIOGRAPHY

- 1 Fahraeus, R., *Acta Med Scandinav*, 1921, iv, 1 The Suspension-Stability of the Blood.
- 2 Popper, M, and Kreindler, F, *Ann. de Méd*, 1925, xvii, 57 La vitesse de sédimentation des hématies Abst in *J Am. Med Assoc.*, 1925, lxxxv, 1925
- 3 Linzenmeier, G, *Arch f Gynäk*, 1920, cxli, 608 Also, *Arch f d ges Physiol*, 1920, clxxi, 169 Untersuchungen über die Senkungsgeschwindigkeit der roten Blutkörperchen
- 4 Oliver, J, and Barnard L, *J Gen. Physiol*, 1924-25, vii, 99 The Influence of Electrolytes on the Stability of Red Blood Corpuscle Suspensions
- 5 Pohle L A., *Radiology*, 1926, vi, 55 Studies on the Suspension Stability of the Human Blood. II The Changes of the Sedimentation Rate of the Erythrocytes in Vitro and in Vivo after X ray Exposure.
- 6 Westergren, A., *Ergebn d inn Med u Kinderh*, 1924, xxvi, 577 Die Senkungsreaktion Allgemein klinische Ergebnisse. Praktische Bedeutung bei Tuberkulose
- 7 Wiltshire, H., *J Path and Bact.*, 1912-13, xvii, 282 An Investigation into the Cause of Rouleau Formation by Human Red Blood Corpuscles
- 8 DeCourcy, J L, *Am J Surg*, 1925, xxxix, 129 Value of Blood Settling Time in Gaiter
- 9 Gnthmann, H., and Schneider, G H., *Arch f Gynäk*, 1925-26, cxvii, 515 Die diagnostische und prognostische Bedeutung der Blutkörperchensenkungsgeschwindigkeit beim Carcinom.
- 10 Gram, H C, *Arch. Int Med*, 1921, xxviii, 312 On the Causes of Variations in the Sedimentation of the Corpuscles and the Formation of the Crusta Phlogistica ("Size," "Buffy Coat") on the Blood.  
Gram, H C, *Acta Med. Scandinav*, 1928, lxxviii, 108 Ueber die Correction der Senkungsreaktion für den Einfluss des Zellvolumenprocentes (Haemoglobin) und über die normalen Grenzen der Senkungsreaktion
- 11 Bönniger, M, and Herrmann, W, *Klin Wchnschr*, 1923, ii, 744 Blutkörperchensenkungsgeschwindigkeit und Volumen
- 12 Rubin, E H, and Smith, N N, *Arch Int. Med*, 1927, xxxix, 303 Relation of Hemoglobin, Cell Count and Cell Volume to the Erythrocyte Sedimentation Reaction
- 13 Hubbard, R. S, and Geiger, H B, *J Lab and Clin Med*, 1928, xiii, 322 Anemia as a Factor in the Sedimentation Time of Erythrocytes.
- 14 Westergren, A., *Acta Med Scandinav*, 1920-21, liv, 247 Studies of the Suspension Stability of the Blood in Pulmonary Tuberculosis.
- 15 Ito, W, *Tohoku J Exper Med.*, 1924, v, 139 Über den Einfluss der Blutgase (des Sauerstoffs und der Kohlensäure) auf die Senkungsgeschwindigkeit der Erythrozyten



# OBSERVATIONS ON THE EFFECT OF VARIOUS FACTORS ON THE DURATION OF THE ELECTRICAL SYSTOLE OF THE HEART AS INDICATED BY THE LENGTH OF THE Q-T INTERVAL OF THE ELECTROCARDIOGRAM

By PAUL D. WHITE AND SEELEY G. MUDD

*(From the Cardiac Clinic and Laboratory of the Massachusetts General Hospital, Boston)*

(Received for publication October 3, 1928)

## INTRODUCTION

The duration of systole, both absolute and relative to diastole, has long been a subject of interest to physiologists, but it has been almost wholly ignored by clinicians. In fact many physicians are not even aware that the duration of systole varies so much as it does with heart rate and that the first and second heart sounds are on some occasions twice as far apart in time as on other occasions. A considerable physiological literature relative to the duration of systole exists, but very little is found in the clinical literature. Fridencia (1) (2) and Miki (32) are the only writers who have discussed the subject in any detail from the clinical point of view, that is, as to the effects of various pathological conditions on the duration of systole. The one finding of common knowledge physiologically and known to the few clinicians who have been interested in the subject is that systole shortens appreciably with increasing pulse rates, but less rapidly than does diastole.

It has seemed to us worth while to gather more data on the effect of various clinical conditions on the duration of electrical systole to supplement the scanty information that we have at present. Hence we have made measurements of electrical systole in a large group of patients with various disease conditions as well as in a group of normal controls.

The duration of electrical systole we have measured from the electrocardiogram (the interval between the beginning of the Q wave

and the end of the T wave) as will be recounted below. Although this interval has not coincided with the measurements made on many mechanical curves, it is possible and in fact probable that it represents more accurately the true duration of systole, provided good measurable curves are obtained, than does any mechanical record. At least this interval is a reliable index of the duration of systole and can be used in a study of the relative effects of various conditions with a high degree of confidence in its reliability for such comparisons.

#### LITERATURE

Fridericia (1) in 1920 published a paper on the duration of systole under normal conditions as measured by the electrocardiogram, which method he considered reasonably accurate. He referred to early measurements of systole both by mechanical records and by electrocardiogram. He concluded from his own study and from observations in the literature that "with rising pulse rates systole and diastole shorten but the latter more." In measuring the Q-T interval of the electrocardiogram, he found the Q or R onset usually sharp with an accuracy of 0.001 second. The end of T was accurate in suitable curves to 0.01 second. If the P wave fell on the T wave, measurement was, he thought, impossible. Variations in the leads were such that he always used lead II. Average errors were not over 0.005 second.

He carefully selected and studied 50 normal people mostly between 20 and 40 years old, 28 male and 22 female. He found the following variations of the duration of systole with pulse rate.

*Table of Fridericia's normal findings (expressed in fractions of a second)*

| Pulse rate | Number of cases | Average Q-T interval | Limits of Q-T interval |
|------------|-----------------|----------------------|------------------------|
|            |                 | <i>seconds</i>       |                        |
| 50-60      | 4               | 0.405                | 0.420-0.398            |
| 60-70      | 10              | 0.370                | 0.402-0.344            |
| 70-80      | 11              | 0.353                | 0.373-0.325            |
| 80-90      | 7               | 0.339                | 0.352-0.327            |
| 90-100     | 8               | 0.335                | 0.360-0.321            |
| 100-110    | 5               | 0.318                | 0.340-0.308            |
| 110-120    | 1               | 0.302                |                        |
| 120-130    | 3               | 0.290                | 0.312-0.273            |
| Over 130   | 1               | 0.280                |                        |

From these data Fridericia constructed the following formula for the determination of the normal average duration of the Q-T interval of the electrocardiogram (systole)  $S = 8.22 \sqrt{p}$ , in which  $S$  = Q-T interval,  $p$  = pulse period in hundredths of a second, and 8.22 = constant.

Fridencia also investigated the effects of various drugs and poisons on the duration of ventricular systole. Among other findings he reported that when the heart rate was increased by adrenalin, systole was shortened much less than when the tachycardia resulted from muscular work.

A later paper the same year (1920) by Fridencia (2) reported his findings in the study of the duration of systole in the electrocardiogram by the use of his formula under various abnormal conditions. He concluded that a deviation greater than 0.045 second from the expected duration of systole as determined by his formula was the outer limit of normal. Two hundred and eleven curves of 124 patients were studied with measurements made from lead II. He found two chief difficulties in measurement, first in arrhythmia, and the 30 cases showing arrhythmia he considered separately, and second in the presence of abnormalities of the T wave (absence, inversion, diphasic nature). He found that inversion of the T wave shortened systole.

Of 94 patients with normal cardiac mechanism, Fridencia found only 65 whose electrocardiograms were satisfactory for measurement. Of these 65 only 7 showed an abnormal duration of systole. The diagnoses in these seven cases were, mitral stenosis 2, aortic insufficiency 1, aortic stenosis 1, aortic and mitral insufficiency 1, myocarditis and scoliosis 1, and chronic nephritis with hypertension and uremia 1. Fridencia concluded that heart muscle weakness and increased work for the heart caused prolonged systole in these cases. However, hypertension, mitral stenosis, and aortic valve disease generally in this series gave normal Q-T intervals.

Of 30 patients with arrhythmia 5 cases of auricular fibrillation were unmeasurable and the T wave was negative in 9 more. In 15 cases with positive T waves all showed normal Q-T intervals except five, too long in four and too short in one. The cases with overlong Q-T intervals showed many extrasystoles in one, complete heart block in two and partial heart block in one. In one case of paroxysmal tachycardia the Q-T interval was found too short but normal in two others with the same mechanism.

Fridencia's papers are of considerable interest and confirm conclusively the observation that electrical systole shortens with increasing heart rates but less than does diastole. The application of his formula to clinical cases and the conclusions drawn at times from rather scanty material demanded further investigation of the subject.

Miki (32) added in 1922 clinical data to Fridencia's observations, but further study has seemed to us necessary to determine whether or not there is any practical application clinically for the determination of the duration of systole from the electrocardiogram.

Miki studied the electrocardiograms of 178 people and applied the three formulas of Fridencia (1), Bazett (30) and Lombard and Cope (26) to them all. There was a great variation in these cases between the various formulas, that of Lombard and Cope being least in agreement. Among the 178 cases there were one of aortic

stenosis, 9 of aortic regurgitation, 10 of mitral regurgitation, 10 of mitral stenosis, 3 of pulmonic stenosis, 12 of combined valve lesions, 20 of left ventricular hypertrophy, 1 of right ventricular hypertrophy, 7 of cardiac dilatation, 7 of "myocarditis," 10 of exophthalmic goiter, 1 of myxedema, 21 of nervousness, 7 with normal hearts and 5 with bradycardia without block. In all this group there were only 13 cases which gave a value of the duration of the Q-T interval more than the limit allowed by Fridericia's formula and these 13 cases were not particularly significant except that most of them had rapid rates (exophthalmic goiter, exercise, nervousness). Of 21 cases of auriculo-ventricular block, 16 gave results longer than the calculated figure from Fridericia's formula while only 9 were greater than Bazett's formula allowed. Miki concluded that in dissociation the duration of systole may become abnormally long and that with damage to heart muscle there tends to be a shortening of systole rather than a lengthening, as Fridericia had believed.

Other workers, mainly physiologists, have as already stated, studied the duration of systole and references to their publications are listed at the end of this paper. It may merely be noted here that the first who made observations on the shortening of systole with increasing pulse rates from pulse tracings were Garrod (3, 4, 5, 6) in 1870 to 1875, Thurston (7) in 1876, Landois (8) in 1876, Waller (9) in 1887, Edgren (10) in 1889, Stockmann (11) in 1889, Kraus (12) in 1891, Ludentz (13) in 1892, Einthoven and de Lint (14) in 1900, and Zuntz and Schumburg (15) in 1901. Since then there have been many papers published concerning the duration of systole as measured from pulse tracings or electrocardiograms, but there has been little of the clinical application of the measurement of systole.

#### METHOD OF PRESENT STUDY

During the past three years the electrocardiograms of 213 individuals, 50 normal and 163 abnormal, have been studied by us to determine the influence of various factors on the duration of electrical ventricular systole. Only good records with clearly defined Q-R-S and T waves were measured. Lead II was generally selected for measurement but if the ventricular deflections were higher and more clearly defined in leads I or III, either of these other leads was used. A comparison of the time intervals in the various leads was made in several cases and as a rule the lead with the highest T wave gave the best indication for measurement of the Q-T interval. Generally such a lead gave also somewhat longer Q-T intervals but usually within the limit of error allowed in measurement.

The duration of electrical systole was considered to be the time interval between the onset of the Q-R-S wave and the end of the T wave. The use of this measurement for the duration of systole has

already been discussed by a number of workers and we believe that even if the Q-T interval may not exactly indicate the duration of systole at least it is a satisfactory index of it and accurate for comparative studies. As we have already remarked we believe that this Q-T interval may actually record the duration of systole better than does any other measurement.

In each case the duration of the Q-T interval was measured for three or more successive heart cycles and then averaged, the heart rate was determined by measurements also of the T-Q intervals giving us thus the total cycle lengths. The Lucas comparator was used for all the measurements. Time intervals were figured in ten thousandths of a second but finally recorded in thousandths of a second. They are accurate we believe down to one-hundredth of a second. The electrocardiograms were made almost invariably in the sitting position, rarely in the recumbent position.

The results have been charted as seen in the figures. Because of the difficulty and dissatisfaction with the use of any formula, simple curves have been constructed to show the normal limits of the duration of electrical systole according to the chief factor influencing it, namely pulse rate. The findings in groups of cases representing a variety of different conditions have been plotted against these normal control curves. We believe that this method serves better than any formula to show when systole is abnormally long or short, and to demonstrate the fact in a simple graphic way. We have plotted in addition to our own 50 normal control cases, 190 normal cases from the literature (those giving satisfactory Q-T measurements), giving us thus a total of 240 normals for comparison with our abnormal cases.

## RESULTS

### *I Normal cases*

*1 Results at rest* In figure 1 is seen the chart of our 50 normal control cases. There is evident a definite relationship between duration of electrical systole in thousandths of a second marked off by horizontal coordinates, and heart rate in beats per minute marked off by vertical coordinates. Thus at rates between 60 and 70 the normal range of systole in our cases was from 0.400 to 0.314 second, at rates between 90 and 100 it was from 0.356 to 0.280, and at rates between

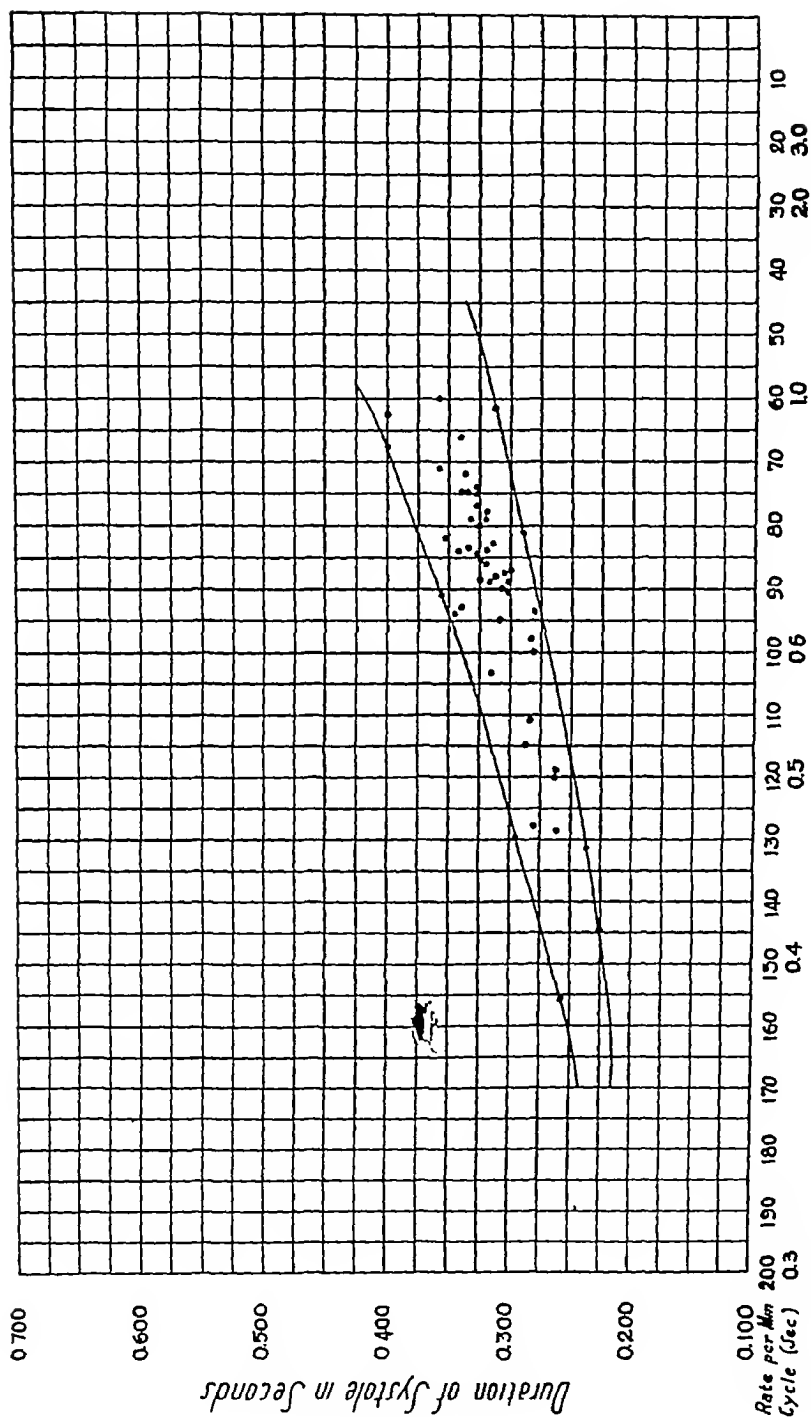


FIG 1 CHART GIVING THE DURATION OF THE Q-T INTERVAL OF THE ELECTRO CARDIOGRAM ACCORDING TO PULSE RATE IN 50 NORMAL SUBJECTS INCLUDING 20 MEN, 15 WOMEN, AND 15 CHILDREN

Each dot represents one case. Heart rate and cycle length are shown in horizontal coordinates and duration of the Q-T interval in thousandths of a second is shown in vertical coordinates. All the succeeding charts are similarly constructed. The limits of measurement of these 50 normal cases of our own series are connected by curves giving a comet shaped track

120 and 150 from 0.295 to 0.225 second. The limits of these measurements are joined, resulting in a comet shaped track, narrow at fast rates. These 50 normal cases included 20 men, 15 women and 15 children (both boys and girls). For this figure and those that follow the duration of the heart cycle is plotted horizontally as well as heart rate, being 2.0 seconds for a rate of 30, 1.0 second for a rate of 60, 0.6 second for a rate of 100, 0.5 second for a rate of 120, and 0.4 second for a rate of 150.

In figure 2 is seen the chart of the 190 normal cases from the literature (Fridericia, (1), Miki (32), Kraus and Nicolai (16), Seham (42), Bazett (30), Katz and Feil (36), and Fenn (35)). They include men, women and children. On this chart in dotted lines are superimposed the curves of figure 1. It will be seen that the limits in figure 2 are somewhat greater through most of the chart than those in figure 1. The coordinates represent as in figure 1 the duration of systole and heart rate. On all the charts to follow, these same coordinates are indicated and the combined limit curves of the 240 normal cases of figures 1 and 2 are superimposed so that at a glance one can tell the situation in a given condition. In the chart of the normal cases from the literature shown in figure 2, the duration of systole at heart rates of 40 to 60 varies from 0.480 to 0.308 second, at rates of 80 to 100 from 0.390 to 0.270 second, at rates of 120 to 130 from 0.312 to 0.230 second, and at rates of 150 to 160 from 0.240 to 0.220 second. Two cases at extremely fast heart rates (180 and 198) fall outside the projected limits, one above and one below.

*2 Normal subjects in different positions.* Five normal subjects were electrocardiographed in three positions—lying, sitting and standing. In every instance with one exception, the pulse rate rose as the position was changed from recumbent to sitting, and in each instance without exception, the pulse rate rose further on changing from the sitting to the standing position. As will be seen in figure 3 the Q-T interval measurements show that the duration of electrical systole varies exactly as does the pulse rate. The normal limit curves from figures 1 and 2 are charted on figure 3.

It would appear that the effect of gravity in cutting down the blood inflow volume in the right heart, as reported by Lombard and Cope (41), is not necessary in these five subjects discussed here to explain

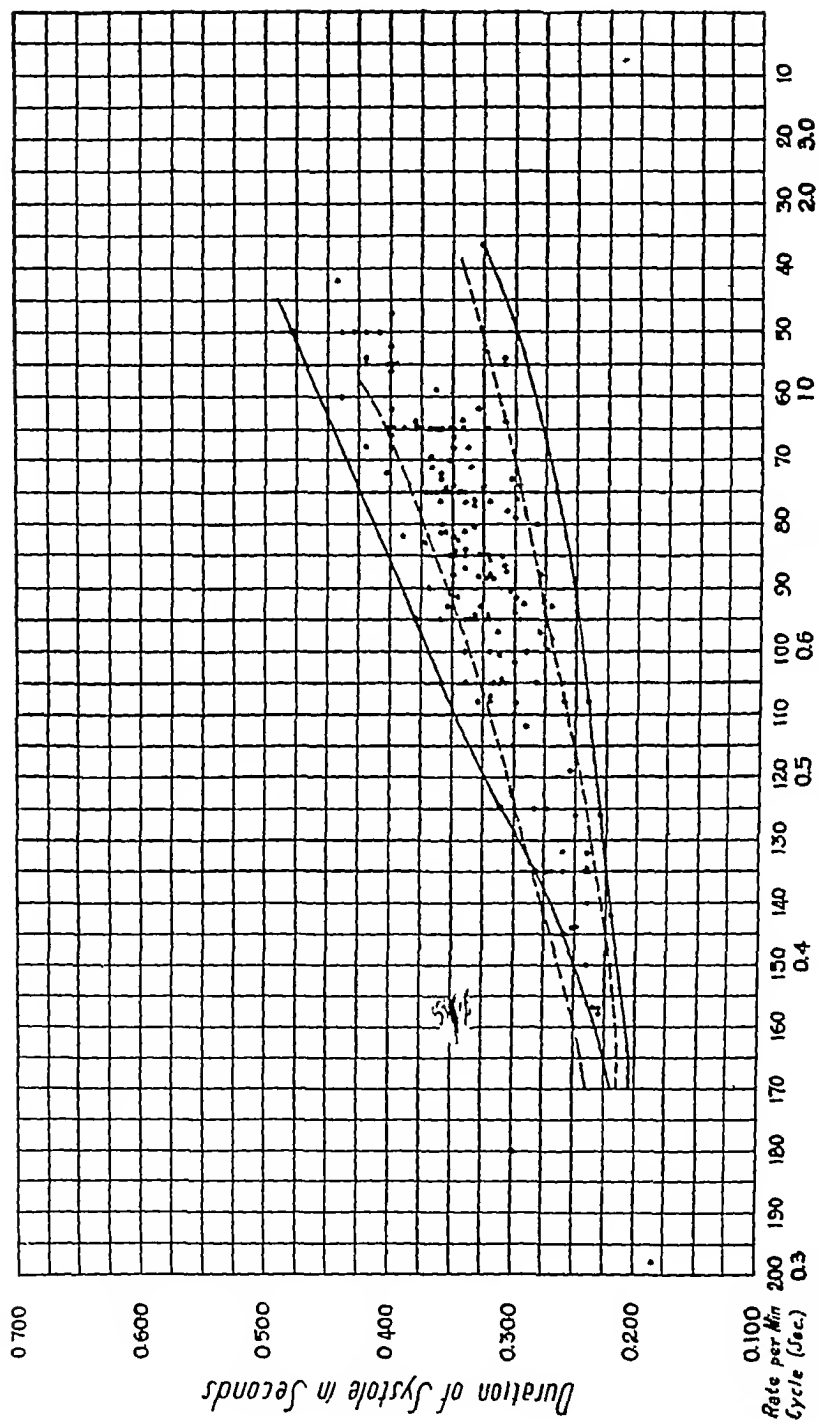


FIG 2 CHART GIVING THE DURATION OF THE Q-T INTERVAL OF 190 NORMAL INDIVIDUALS PUBLISHED IN THE LITERATURE. The outer limits of measurement of these cases are connected by solid lines and the limit lines of our own series of 50 normal cases are superimposed as interrupted lines. Two cases at very rapid rates are seen to fall outside these limit lines when projected.

In all figures to follow the combined outer limit lines of these 238 normal cases are charted and are used as an indication of normal or abnormal length of Q-T interval at varying heart rates.

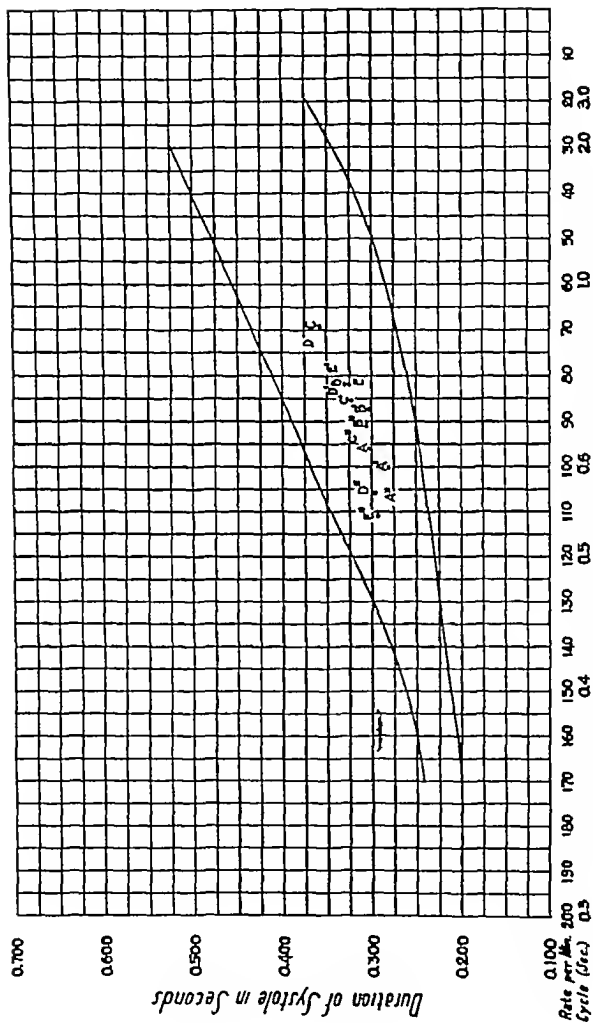


FIG 3 THE Q-T INTERVALS OF FIVE NORMAL SUBJECTS IN VARIOUS POSITIONS

A-E, in lying position, A'-E' in sitting position, and A''-E'' in standing position

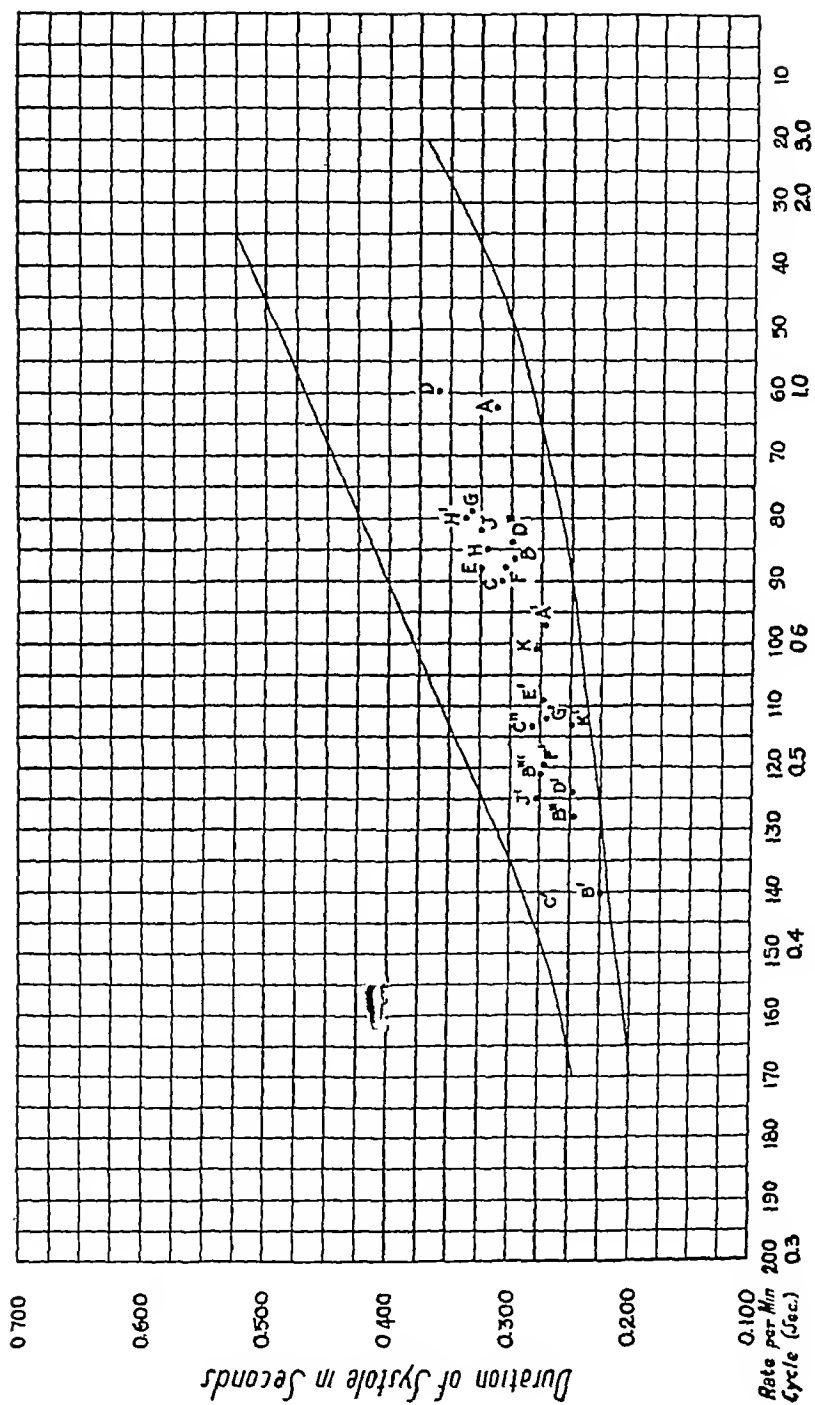


FIG 4 THE Q-T INTERVALS OF TEN NORMAL INDIVIDUALS BEFORE AND AFTER EXERCISE  
A-K, at rest, A'-K', B''-D'', B''' after exercise

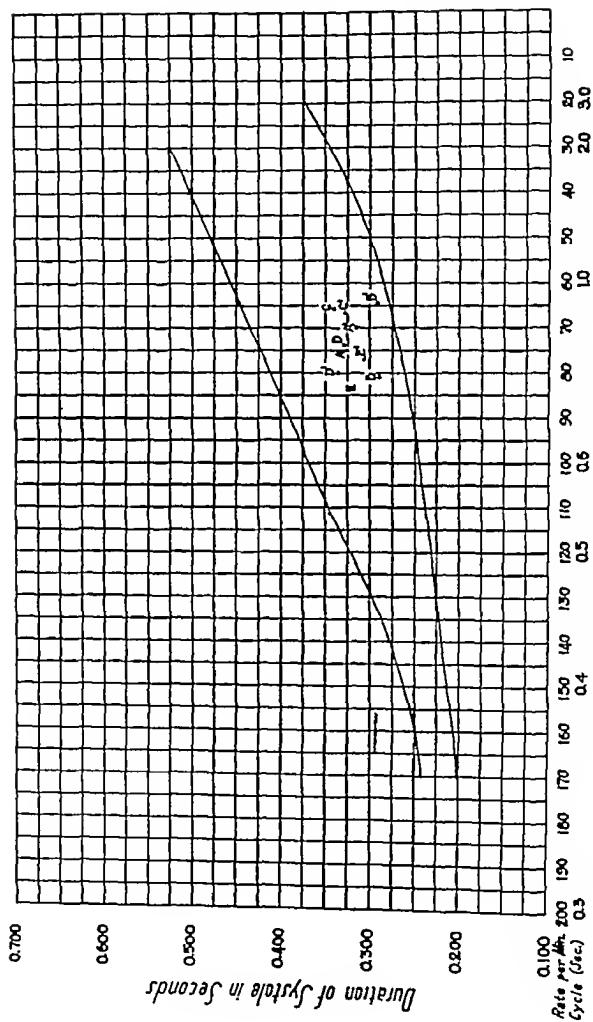


FIG 5 THE Q-T INTERVALS OF FIVE NORMAL INDIVIDUALS BEFORE AND AFTER DIGITALIZATION

A-E, before digitalis, A'-E' after digitalis

the variation in the duration of electrical systole. Heart rate alone as in the records from the larger series of normal subjects in the sitting position (fig 1) may be accounted the chief, though doubtless, not the sole factor. Whatever part gravity may play in these five subjects is a minor one. However, it is quite possible that heart rate itself acts by its effect on the amount of blood entering the heart, duration of electrical systole being controlled by this content of blood rather than by the varying heart rate in itself. Other things being equal, the faster the heart rate the less blood enters the heart, the slower the rate the larger the blood volume in the ventricles. If this is so it would appear that gravity too, with change in position, should influence the duration of systole, but this influence is not apparent in the measurements of the present five subjects.

3 *Normal cases after exercise* In figure 4 are charted the results of exercise on the duration of ventricular systole in 10 normal subjects (young men). First, the subjects were electrocardiographed at rest (A to K) and then immediately after exercise consisting of running up and down four flights of stairs (A' to K'). Three subjects were recorded a second time after exercise (B'', C'', D'') and one was recorded a third time (B''')

It is obvious that all the measurements remained well within the normal limits as charted from figures 1 and 2.

4 *The effect of digitalis on normal subjects* Five normal subjects were digitalized (to mild toxic effects) in the course of 7 to 10 days (20 to 30 grams of digitalis leaf by mouth), and electrocardiograms before digitalis and at the height of the effect of the drug were measured. As will be seen in figure 5, all measurements were well within normal limits permitting us to conclude that digitalis in the normal subject does not influence appreciably the duration of electrical systole as measured by the Q-T interval of the electrocardiogram.

## II Abnormal cases

We now turn to strictly pathological conditions and will consider first structural defects such as cardiac enlargement and valvular disease, then the effect of other conditions and diseases like hypertension per se and diabetes, and finally functional conditions like congestive failure and abnormal tachycardias, bradycardias, and

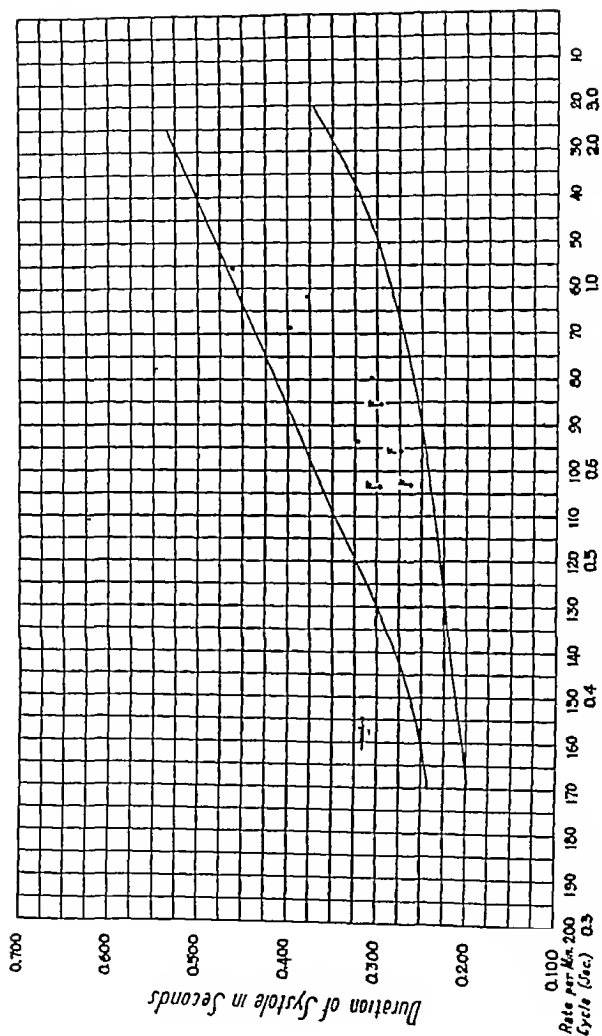


FIG 6 THE Q-T INTERVAL OF TEN PATIENTS WITH VERY LARGE HEARTS WITHOUT ABNORMAL AXIS DEVIATION

F = four cases with auricular fibrillation also

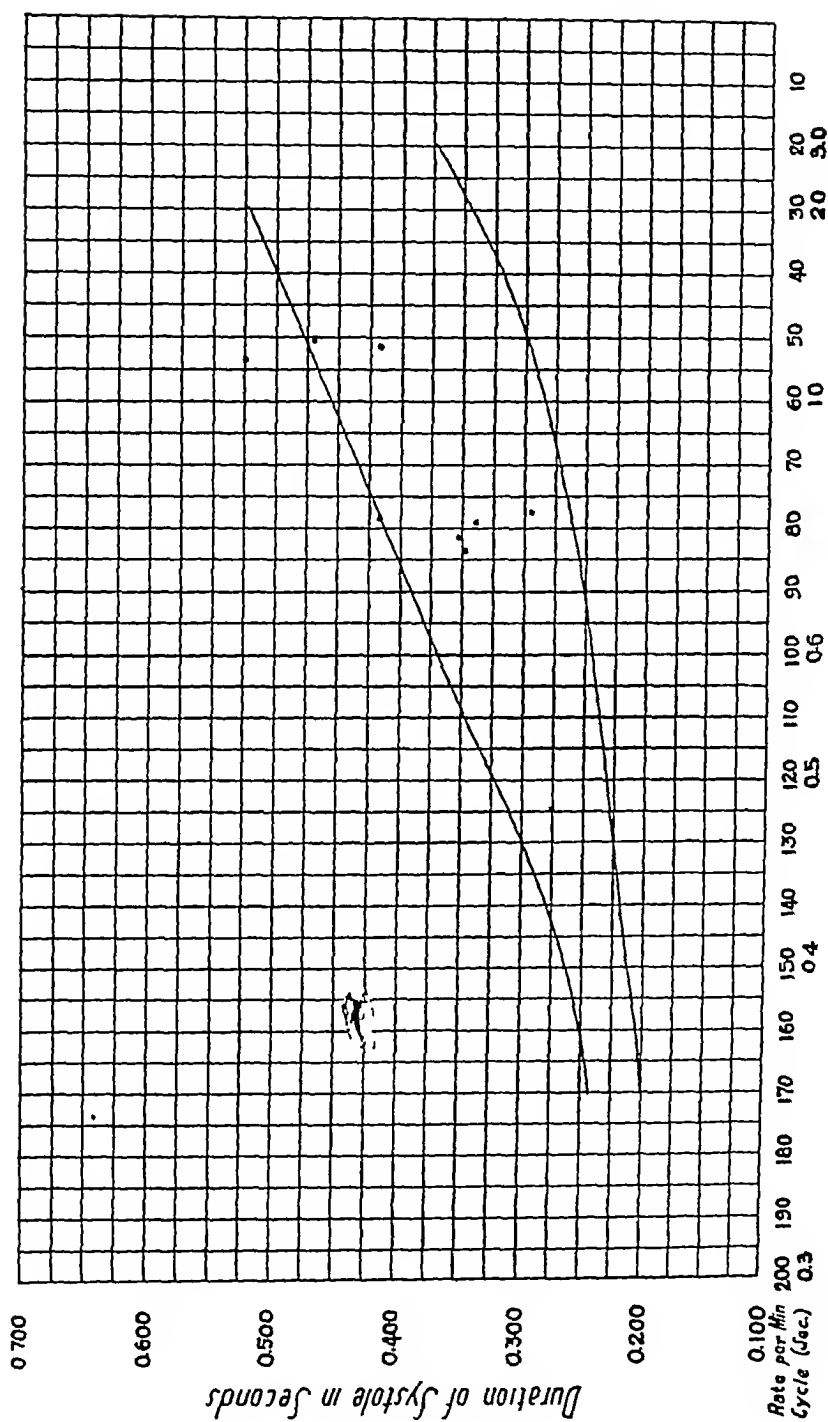


FIG 7 THE Q-T INTERVALS OF NINE PATIENTS WITH VERY LARGE HEARTS AND ABNORMAL LEFT AXIS DEVIATION

arrhythmia There are included in these pathological groups a total of 163 cases

*1 Structural A Enlargement of the heart without abnormal axis deviation* Ten cases with marked cardiac enlargement but without abnormal axis deviation were studied In some, the cause of the enlargement was unknown In a few, hypertension or aortic regurgitation were factors Six had normal rhythm and four had auricular fibrillation The results are charted in figure 6 and the Q-T interval is within normal limits in every instance.

We had thought that marked enlargement of the heart might cause prolongation of electrical systole This proved not to be the case when the Q-T intervals of this group of cases were measured

*B Enlargement of the heart with abnormal left axis deviation* To supplement the cases of cardiac enlargement without abnormal axis deviation, nine more cases with enlarged heart and an abnormal degree of left axis deviation were studied Hypertension and aortic regurgitation were the chief underlying factors Only one of these nine patients showed a Q-T interval longer than the normal outer limits (fig 7) One other case was on the upper border line of normal and one just within it The others were well inside the normal limits

Thus of 19 cases with very large hearts, only one showed a duration of systole above the normal limits as measured by the Q-T interval It may be said, therefore, that cardiac enlargement does not ordinarily cause an increase in the Q-T interval

*C Aortic regurgitation* Nine cases of aortic regurgitation, due to rheumatic or luetic etiology, were studied and are charted in figure 8 In all the duration of systole was normal

*D Aortic stenosis* Aortic stenosis, both in animal experimentation and in the clinic, has been reported as a cause of systolic lengthening (13, 20, 37, and 38) Figure 9 shows the results of the measurement of electrical systole of 6 cases of our own, one of which, very marked in degree, was confirmed by post mortem examination All measurements fall within normal limits, including even the one case B with slight bundle branch block in addition

*E Mitral stenosis* Ten cases of mitral stenosis all showed Q-T intervals well within normal limits (fig 10)

*F Congenital heart disease* Eight cases of congenital cardiac

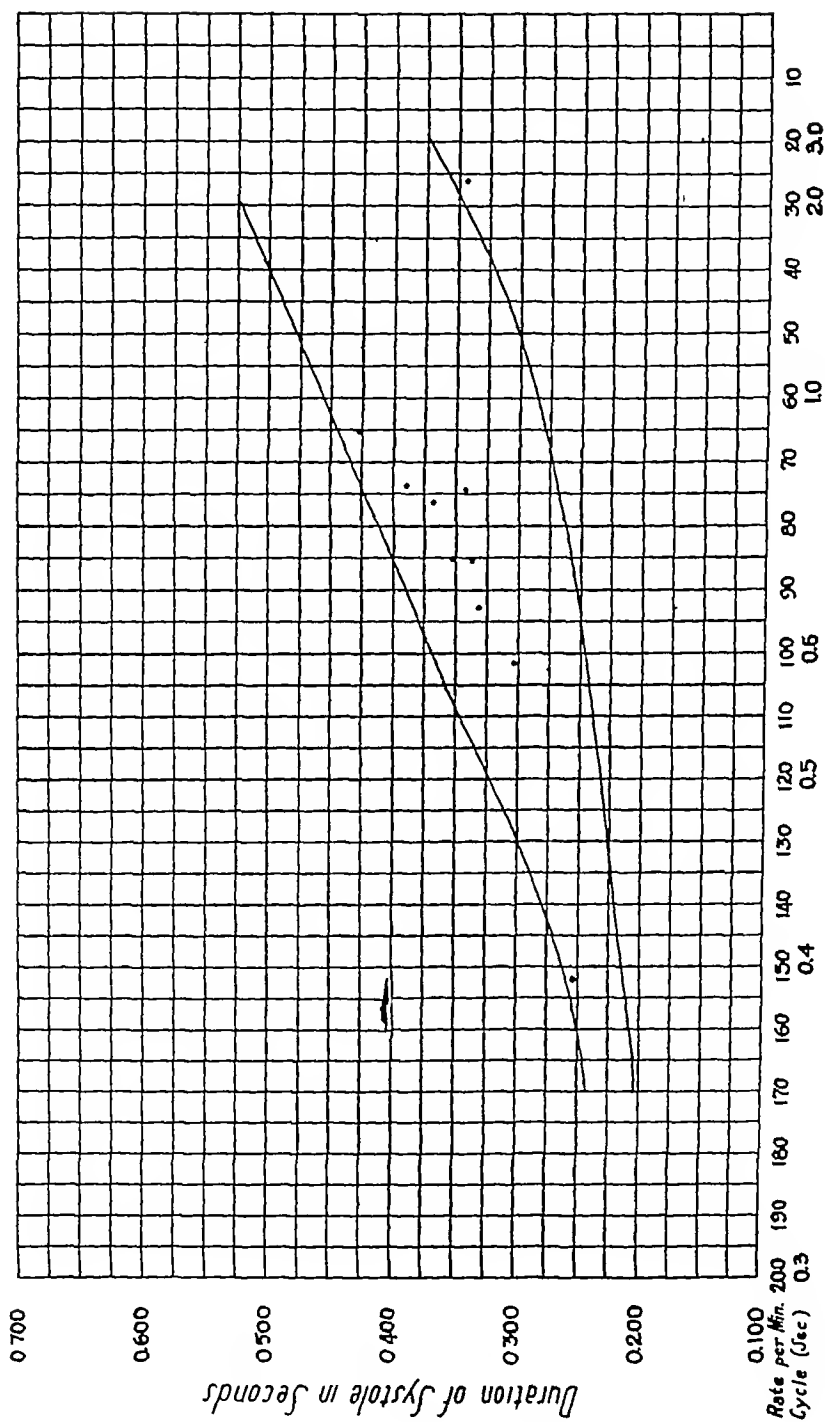


FIG 8 THE Q-T INTERVALS OF TEN PATIENTS WITH AORTIC REGURITATION

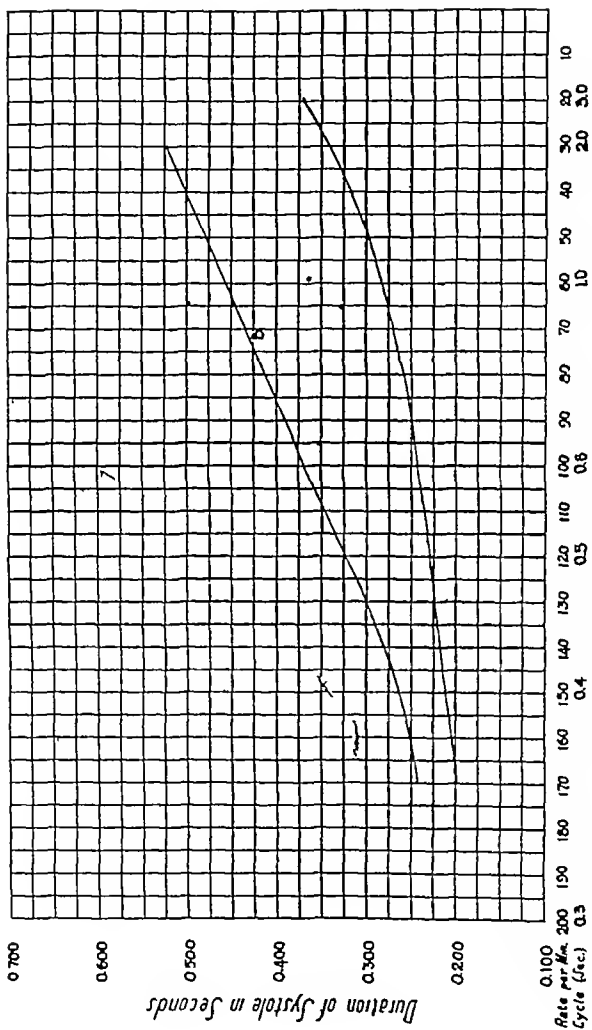


FIG 9 THE Q-T INTERVALS OF SIX PATIENTS WITH AORTIC STENOSIS, ONE CASE B HAD ALSO RIGHT BUNDLE BRANCH BLOCK

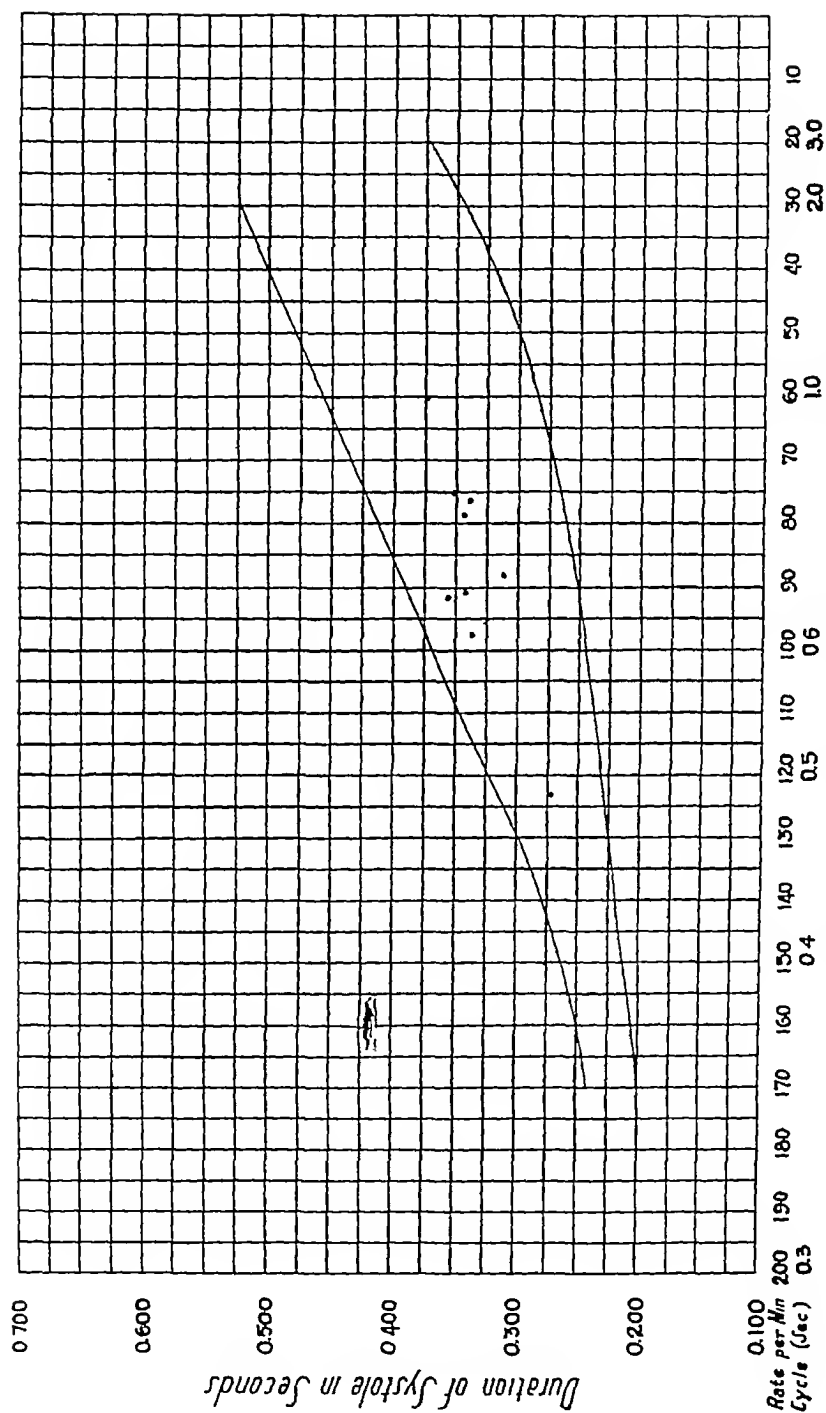


FIG 10 THE Q-T INTERVALS OF TEN PATIENTS WITH MITRAL STENOSIS

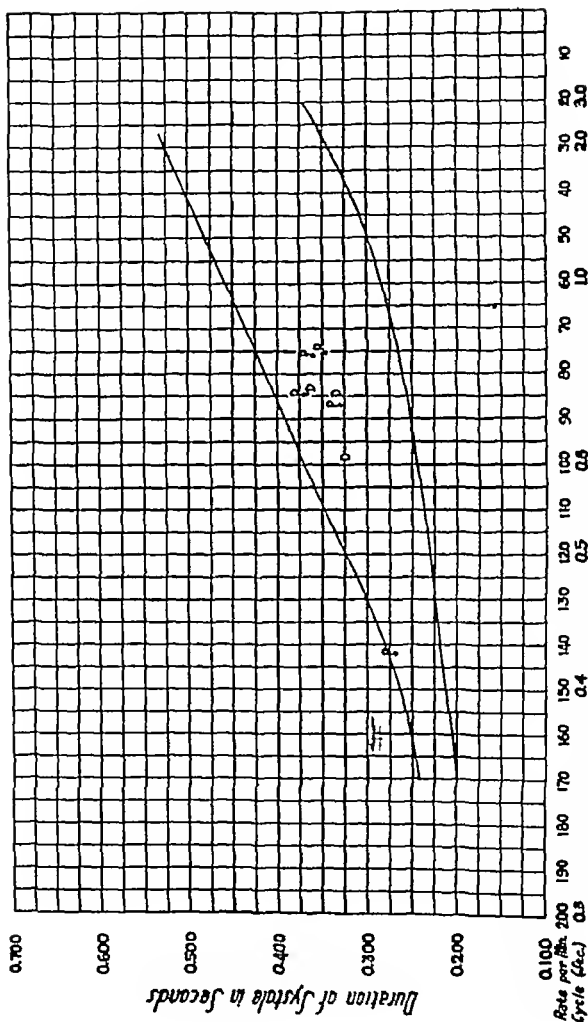


FIG 11 THE Q-T INTERVALS OF EIGHT PATIENTS WITH CONGENITAL HEART DISEASE  
 D = those with patent ductus arteriosus (3) P = those with pulmonic stenosis (5)

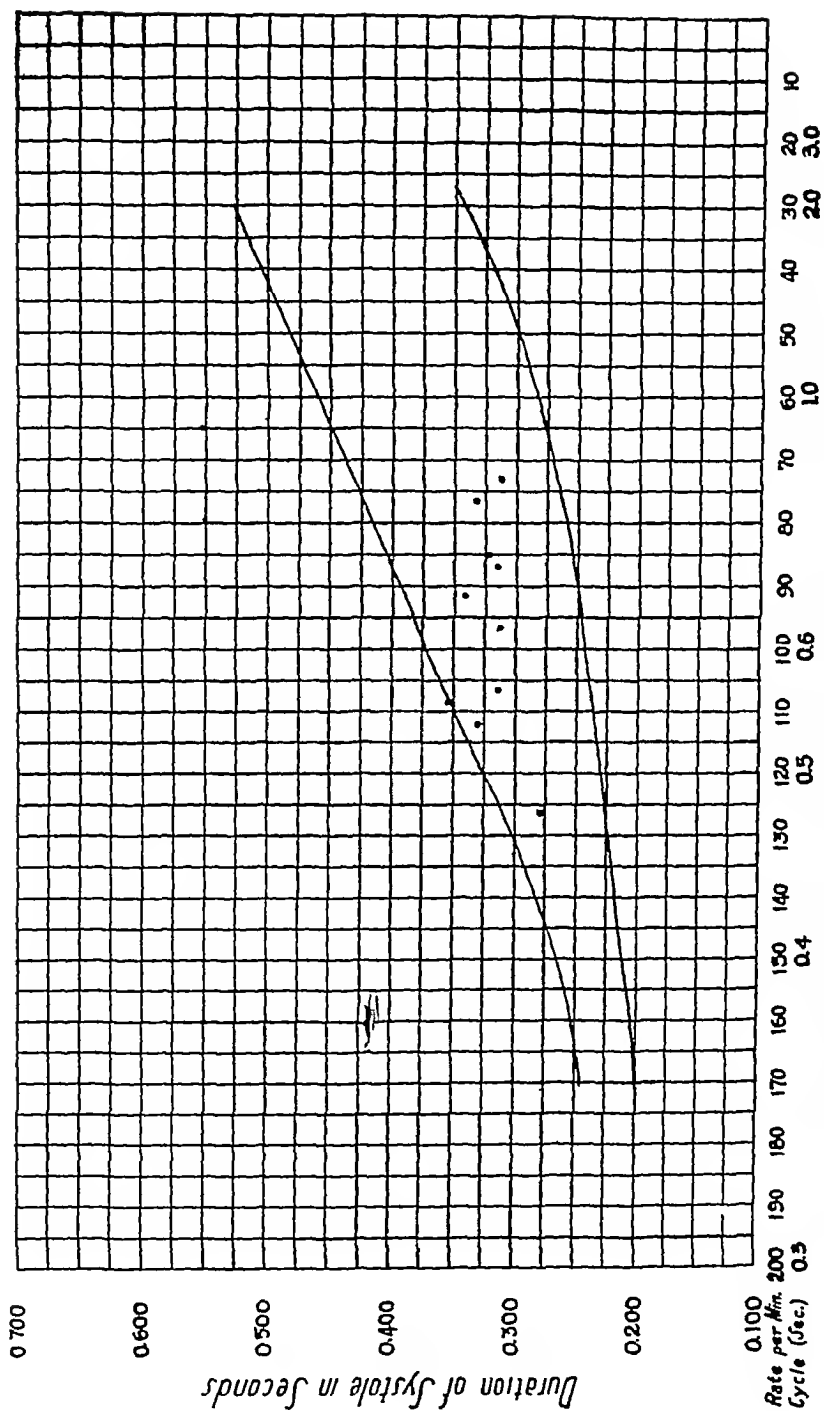


FIG 12 THE Q-T INTERVALS OF TEN CASES WITH UNCOMPLICATED ESSENTIAL HYPERTENSION

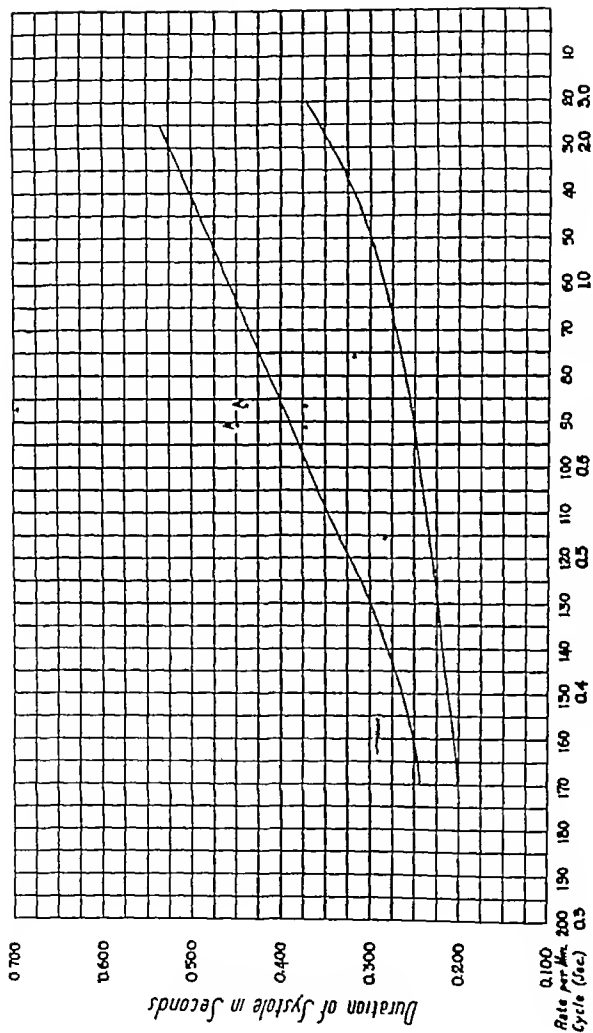


FIG 13 THE Q T INTERVALS OF FIVE CASES WITH UREMIA

A = 2 records of one case



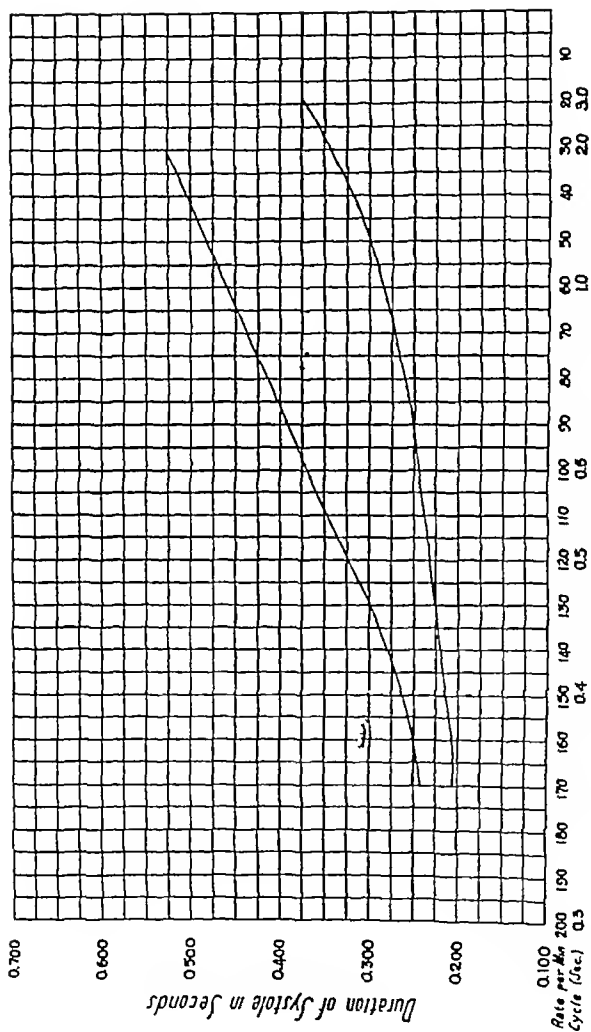


FIG 15 THE Q-T INTERVAL OF FIVE PATIENTS WITH DIABETES MELLITUS

defects all showed normal duration of systole as measured by the Q-T interval. Five of the patients were diagnosed as having pulmonic stenosis (*P*) and three as having patent ductus arteriosus (*D*).

*2 Extrinsic factors A Hypertension* Hypertension has at times past been found present when systole is prolonged (28, 35 and 37) but in ten cases that we have studied in which the single factor of hypertension entered, we found all with a Q-T interval within normal limits, though one was on the upper limit of normal. These cases showed little or no cardiac enlargement by physical examination or x-ray and no abnormal axis deviation by electrocardiogram.

*B Uremia* Five cases of uremia showed normal duration of electrical systole in all except one (fig 13). On both of two separate occasions, this patient's Q-T interval was too long.

*C Blood calcium studies* As shown by figure 14, the content of calcium in the blood serum appears definitely to be a factor in controlling the duration of the Q-T interval, in almost as striking a manner as is the pulse rate. This relationship has already been pointed out by Carter and Andrus (34) and our findings are strongly confirmatory. Five cases have been studied by us from the standpoint of calcium content of the blood serum and the two cases (tetany) which showed marked diminution of the serum calcium (A with 4.0 mgm and D with 5.0 mgm per 100 cc of blood) both showed prolongation of the Q-T interval beyond the normal, with a return to normal as the blood calcium rose.

*D Diabetes mellitus* Five cases of diabetes mellitus were studied by us, three of them during the stage of hyperglycemia, all showed Q-T intervals within normal limits (fig 15).

*E Hyperthyroidism* The effect of hyperthyroidism was studied in eleven patients, in two of whom electrocardiograms were taken before and after correction of the hyperthyroidism by operation. Also in two of the cases, records were obtained during periods of auricular fibrillation as well as during normal rhythm. As will be seen in figure 16, all the measurements are within normal limits except two, one a case with a metabolic rate of plus 32 per cent and normal rhythm and the other after operation with a metabolic rate of -5 per cent and auricular fibrillation. This latter case showed during normal rhythm, normal Q-T intervals both before and after operation. All the other cases showed normal duration of electrical systole.

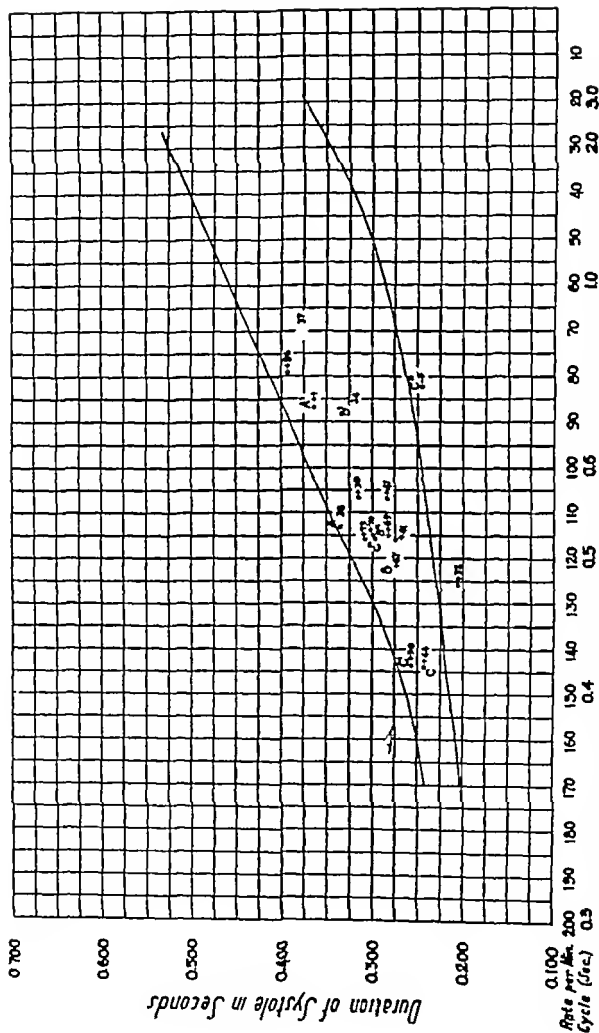


FIG 16 THE Q-T INTERVALS OF ELEVEN PATIENTS WITH HYPERTHYROIDISM, IN TWO OF THESE CASES A AND C THE Q T INTERVALS AFTER OPERATION AND CORRECTION OF THE HYPERTHYROIDISM ARE ALSO SHOWN (A', C', C'')

B'', C'' = cases during auricular fibrillation The figures below the dots give the basal metabolic rates

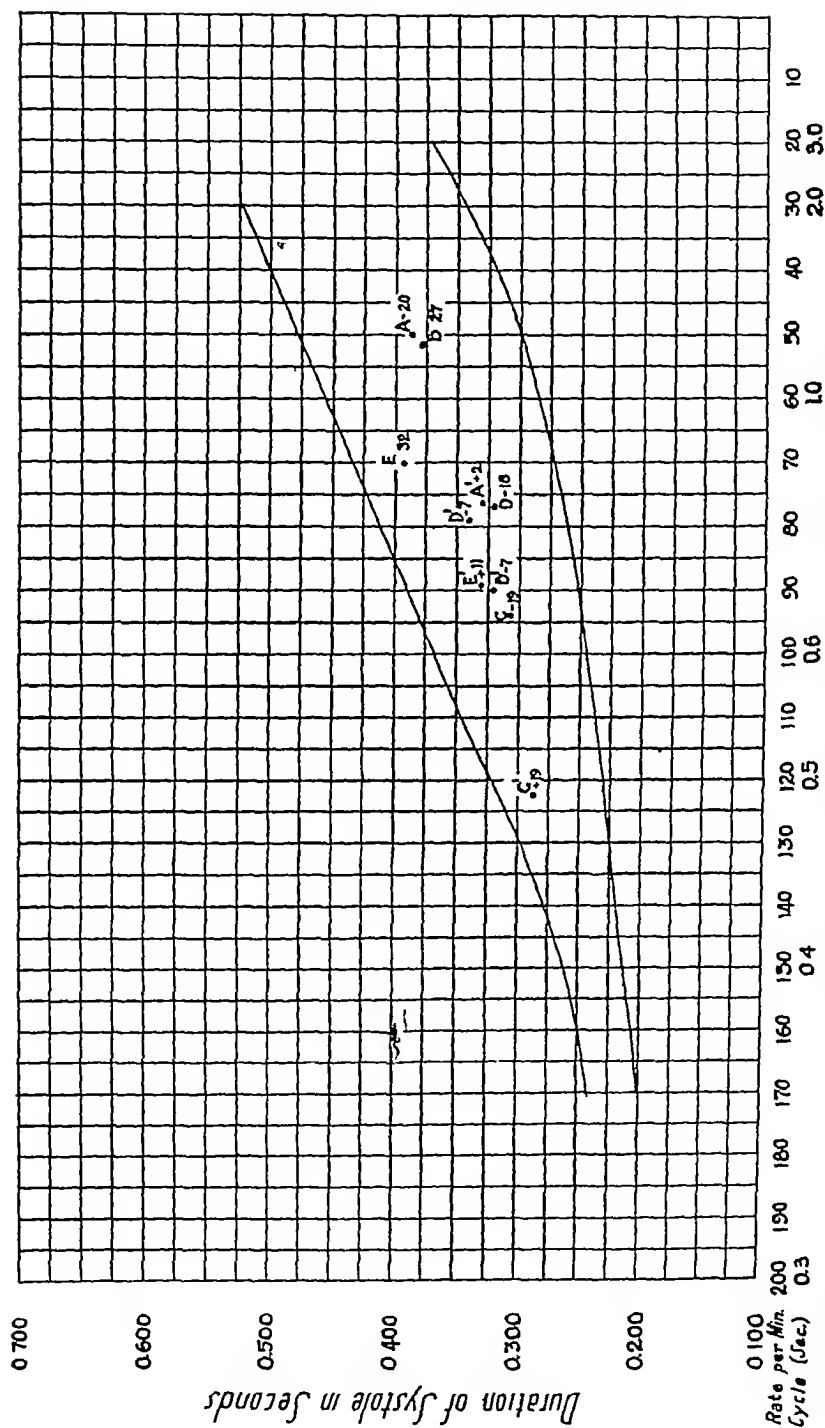


FIG 17 THE Q-T INTERVALS OF FIVE CASES WITH HYPOTHYROIDISM A-E

The Q-T intervals of the same cases after thyroid therapy are also given (A'-E') The basal metabolic rates are shown by figures below the dots representing the Q-T intervals

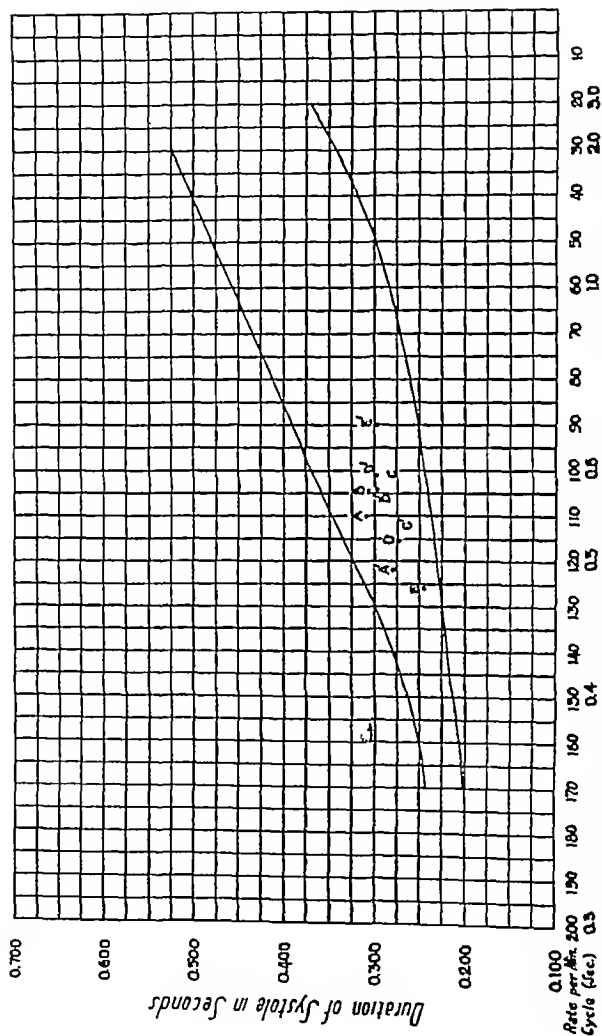


FIG 18 THE Q-T INTERVALS OF FIVE PATIENTS DURING (A-E) AND AFTER (A'-E') ACUTE INFECTION

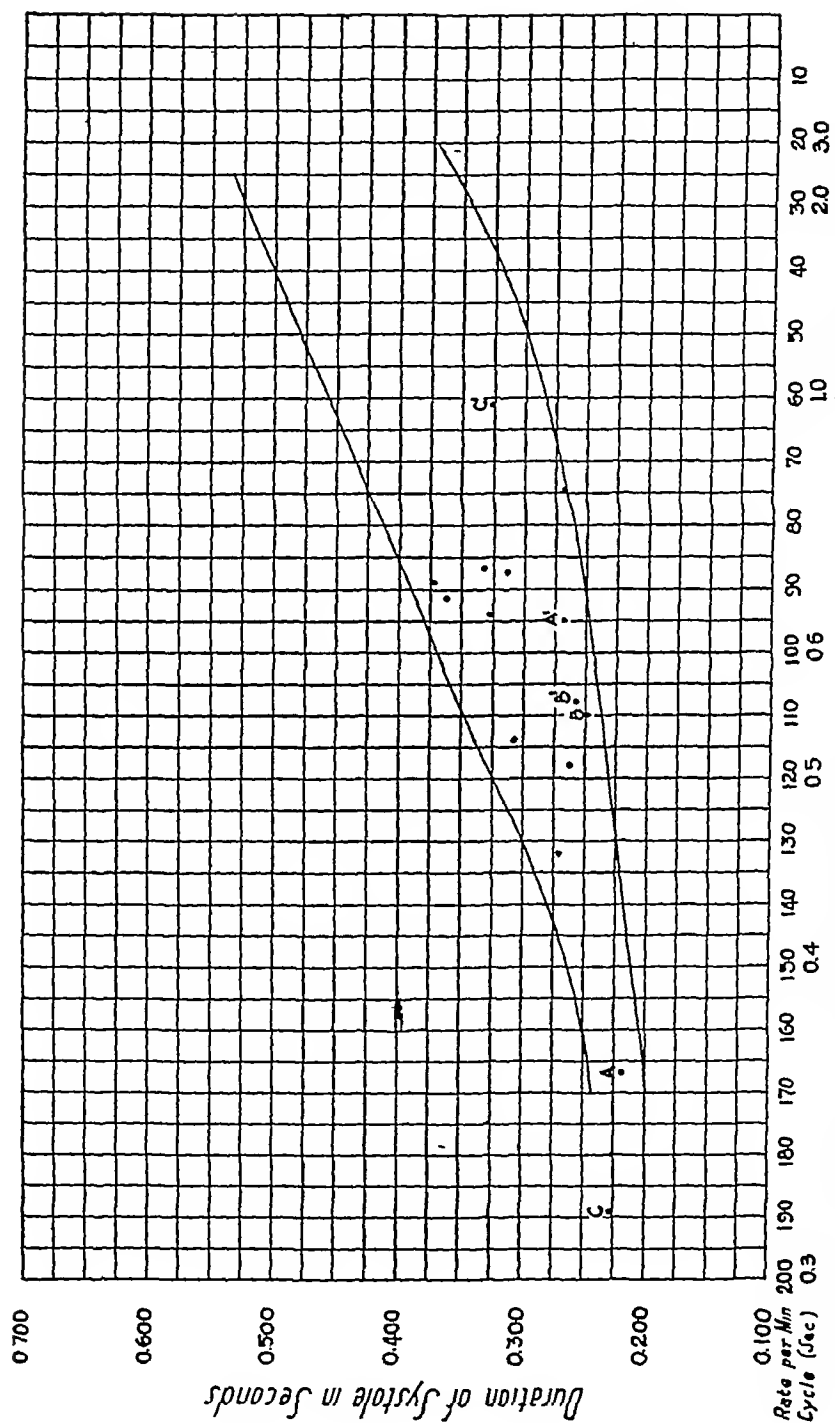


FIG 19 THE Q-T INTERVALS OF TWELVE PATIENTS WITH CONGESTIVE FAILURE, IN THREE CASES (A, B AND C), THE Q-T INTERVALS AFTER RECOVERY ARE ALSO SHOWN (A', B' AND C')

Auricular fibrillation additionally present in cases A and C

*F Hypothyroidism* The effect of hypothyroidism on the duration of the Q T interval was measured in five cases both before and after thyroid therapy. In every case, the findings were well within normal limits as seen in figure 17, although we had thought that a sluggish heart in myxedema might prolong electrical systole. Of course, the pulse rate was invariably slower during the hypothyroid stage, and in accord with the slower pulse rate, the Q-T interval was longer.

*G Acute infection* Five cases have been studied to determine whether or not acute infection affects the duration of the Q-T interval. Electrocardiograms were taken and measured both during the infection and after recovery. Pneumonia was the infection in two cases and tonsillitis and pharyngitis in the other three. The Q T interval during the infection was generally short but in exact relationship to the degree of tachycardia and hence well within normal limits.

It is of considerable interest to observe that in two of the cases, the pulse rate was actually higher after the infection than during it, due to excitement and effort syndrome but nevertheless the Q T intervals varied consistently as the heart rate.

*3 Functional conditions A Congestive failure* To determine whether congestive failure affects the duration of electrical systole we studied twelve cases, three with auricular fibrillation and nine with normal rhythm. Three of these patients we investigated again after the congestive failure had disappeared under rest and digitalis. We have already noted that digitalis per se does not affect the Q-T interval. All the measurements both during and after congestive failure were within normal limits as shown in figure 19.

Heart muscle weakness and congestive failure have been mentioned in the literature as possible causes for prolongation of systole (2, 29) and also for shortening of systole (32, 37). Our study of the twelve cases reported above shows no definite effect either one way or the other.

*B Auricular paroxysmal tachycardia* Five cases of auricular paroxysmal tachycardia have been studied by us both during the paroxysms and also during normal rhythm. A little difficulty has been experienced at times in making the measurements but only those electrocardiograms allowing a reasonable degree of accuracy have been selected. As seen in figure 20, there is a tendency for the Q-T

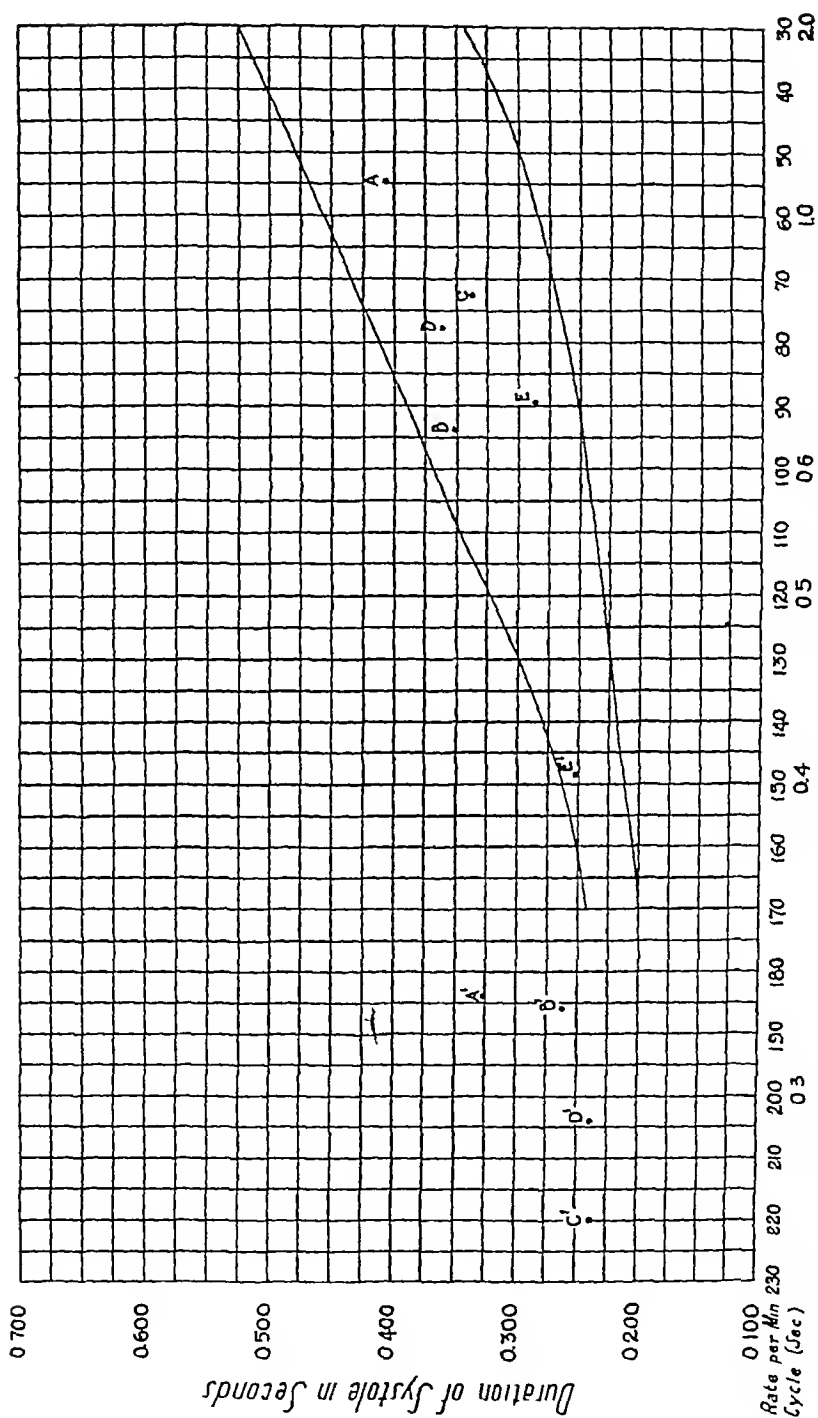


FIG 20 THE Q-T INTERVALS OF FIVE PATIENTS DURING AURICULAR PAROXYSMAL TACHYCARDIA (A'-E') AND DURING NORMAL RHYTHM (A-E)

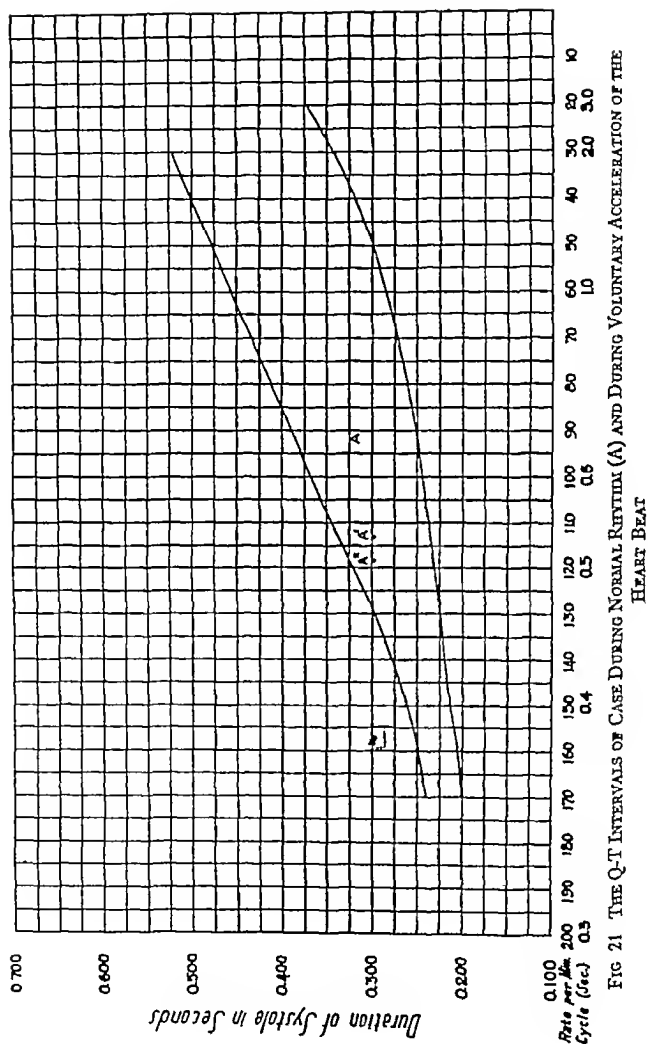


FIG 21 THE Q-T INTERVALS OF CASE DURING NORMAL RHYTHM (A) AND DURING VOLUNTARY ACCELERATION OF THE HEART BEAT

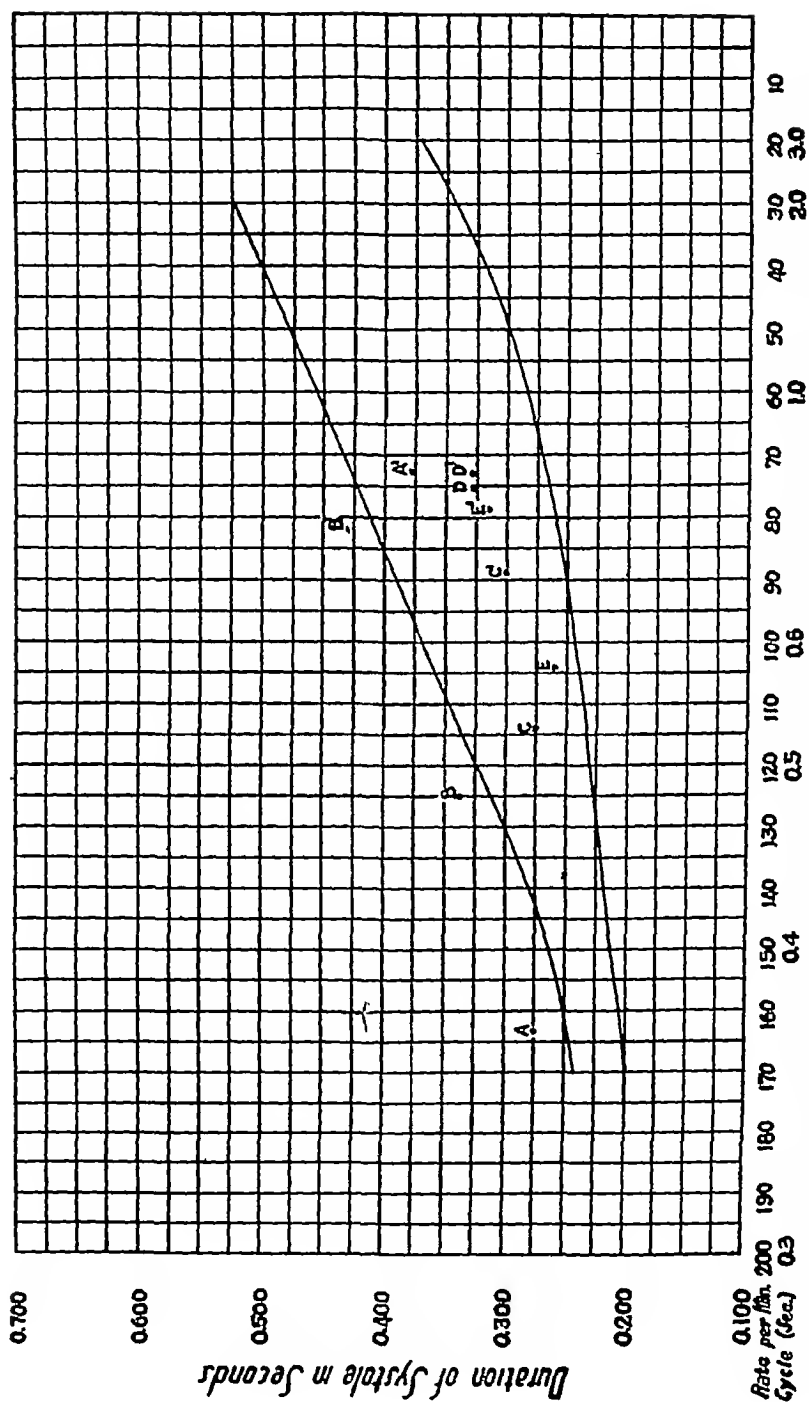


FIG 22 THE Q-T INTERVALS OF FIVE PATIENTS WITH AURICULAR FIBRILLATION (A-E) AND OF THE SAME PATIENT AFTER RESTORATION TO NORMAL RHYTHM BY QUINIDINE SULPHATE (A'-E')

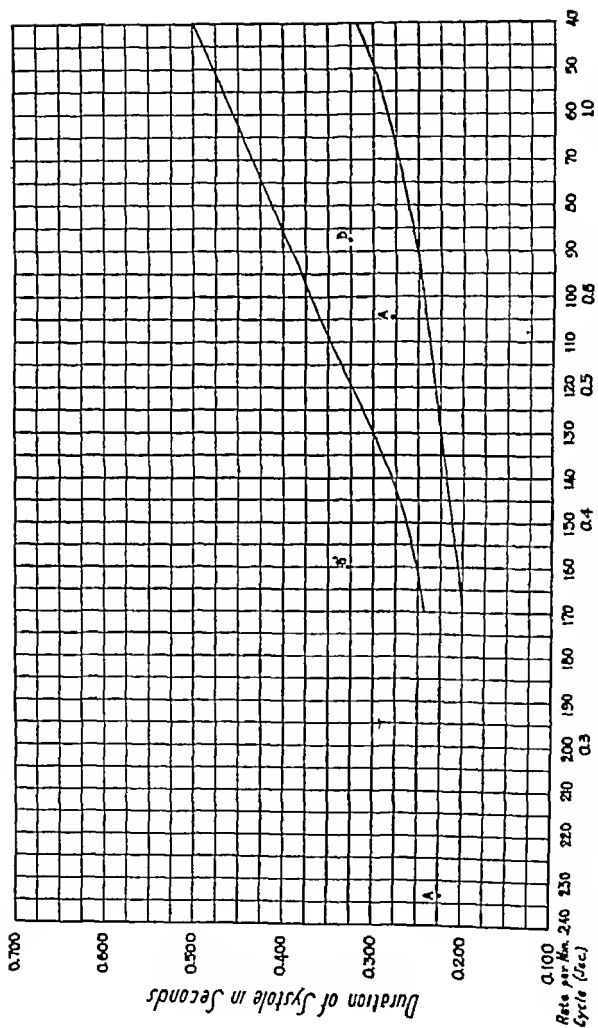


FIG 23 THE Q-T INTERVALS OF TWO CASES OF VENTRICULAR PAROXYSMAL TACHYCARDIA DURING THE PAROXYSMS (A', B') AND DURING NORMAL RHYTHM (A, B)

interval to be longer at the paroxysmal rates than would be expected on continuing the so-called normal limit curves. The slowest paroxysmal rate—148—was found with a Q-T interval within normal limits, but all four cases with rates over 180 showed relatively long Q-T intervals. Of course, here we have no normal controls for comparison.

*C Voluntary acceleration of the heart rate* One rare case of voluntary acceleration of the heart rate was studied and found to have durations of the Q-T interval perfectly normal at varying rates (fig 21).

*D Auricular fibrillation with restoration of normal rhythm* The effect of auricular fibrillation on the duration of the Q-T interval was studied in five cases in whom normal rhythm was later restored by quinidine sulphate. A comparison of the Q-T interval during auricular fibrillation and during normal rhythm was made. The results are charted in figure 22. All measurements were normal except three which were prolonged, two of these measurements were from the same case (B B') during auricular fibrillation and during normal rhythm and that case had also right bundle branch block which will be discussed later, the third measurement was also delayed beyond the normal but occurred in a case only during a very rapid ventricular rate (164) in auricular fibrillation, the Q-T interval being within normal limits when the rhythm was normal and the rate slow (73). The other three cases showed entirely normal rhythm. It would seem then from these measurements and those of seven other cases with auricular fibrillation shown in figures 6 and 19, that auricular fibrillation in itself does not affect the Q-T interval, which may be prolonged, however, if there is a coincident bundle branch block or an extreme tachycardia. Auricular fibrillation has been reported in some cases in the literature with overlong Q-T intervals (2) and also with Q-T intervals shorter than normal (2, 31, 32, 36).

*E Ventricular paroxysmal tachycardia* Two cases of ventricular paroxysmal tachycardia were electrocardiographed during their paroxysms and during normal rhythm. The results are shown in figure 23. In one case the Q-T interval is much prolonged beyond the normal outer limit (B') and in the other case the Q-T interval falls also above this upper limit line if projected, but only a little above normal. In both cases the Q-T interval is well within normal limits during normal rhythm.

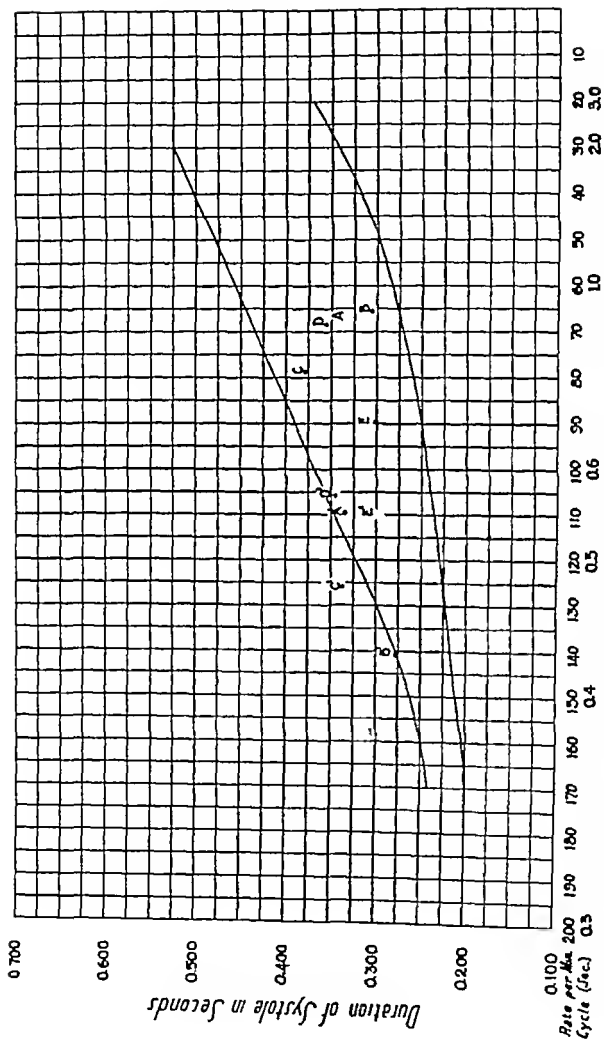


FIG 24 THE Q-T INTERVALS OF AURICULAR PREMATURE BEATS (A'-E') AND OF NORMAL BEATS (A-E) IN FIVE CASES

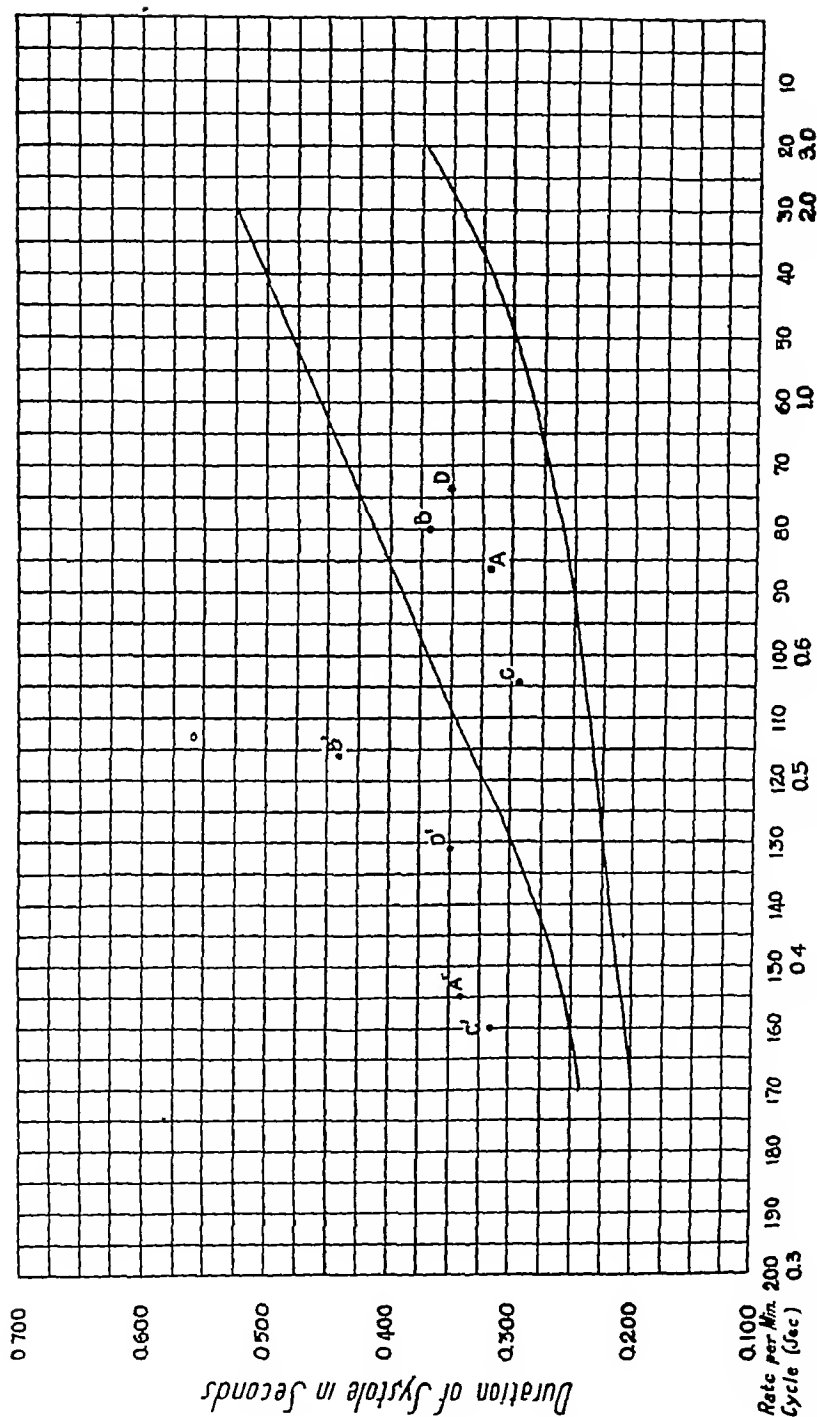


FIG 25 THE Q-T INTERVALS OF VENTRICULAR PREMATURE BEATS (A'-D') AND OF NORMAL BEATS (A-D) IN FOUR CASES

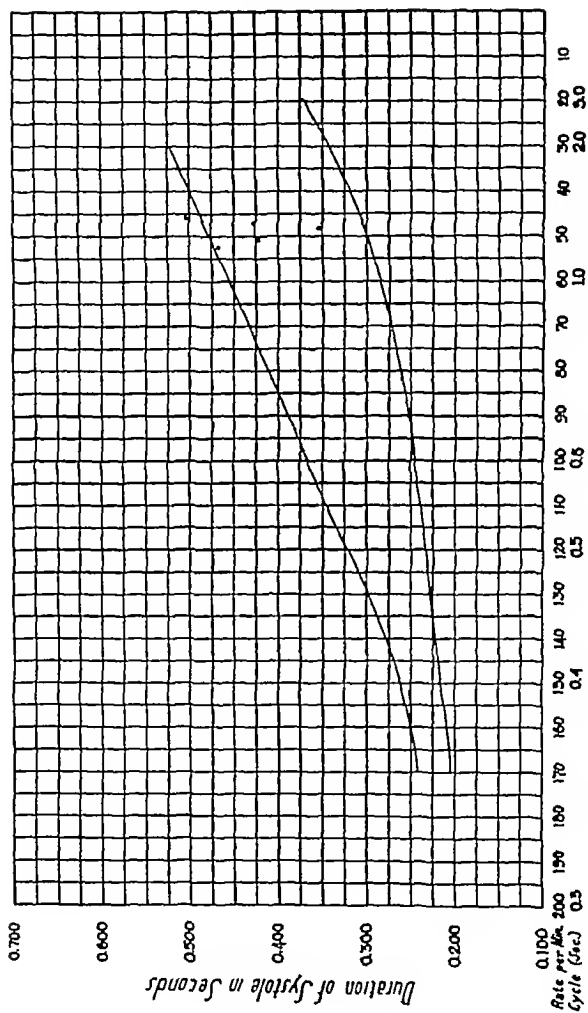


FIG 26 THE Q-T INTERVALS OF SEVEN CASES OF SINOAURICULAR BRADYCARDIA

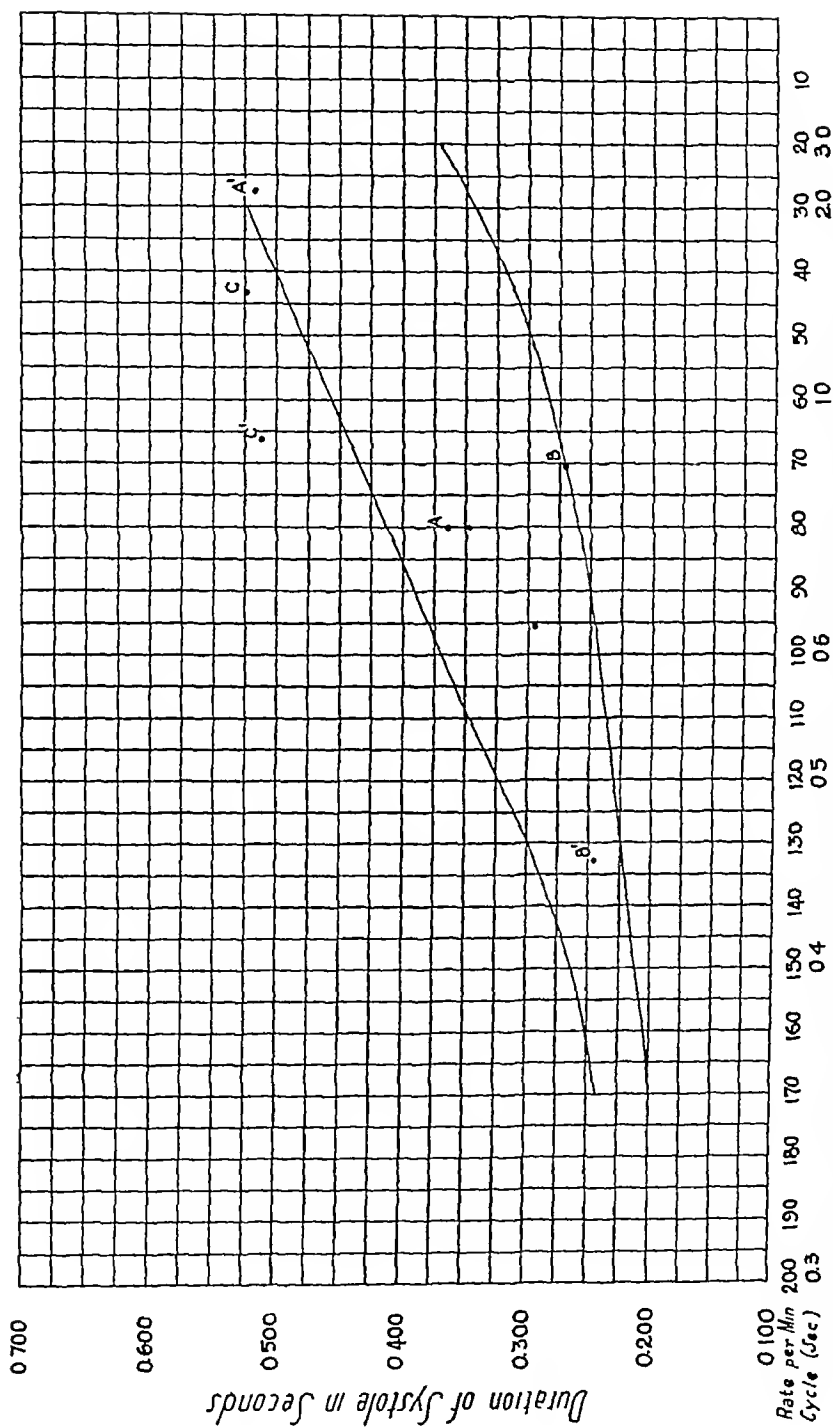


FIG 27 THE Q-T INTERVALS OF FIVE PATIENTS WITH PARTIAL AURICULO-VENTRICULAR BLOCK

A-A', B-B', and C-C', represent the Q-T intervals in three of the cases at varying degrees of block and heart rate

*F Auricular premature beats* A comparison has been made in five cases between the duration of the Q-T interval of an auricular premature beat without aberration and that of a normal beat (fig 24). In one case the auricular premature beat Q-T interval was beyond the upper normal limit and in three other cases on or very close to it. Only one was well within normal limits. The "rate" for the premature beats was calculated from the interval between the preceding normal beat and the premature beat.

*G Ventricular premature beats* A similar study was made of four cases of ventricular premature beats. Figure 25 shows that in all of the four cases the Q-T interval of the premature beat was much prolonged beyond the normal limits for heart rates based on the interval between the preceding normal beat and the premature beat. This is what one would expect since the abnormal origin of the beat should cause a slower spread of the impulse and slower rise of intraventricular pressure than normally.

*H Sinoauricular bradycardia* Seven instances of sinoauricular bradycardia without heart disease were studied. All Q-T intervals were within normal limits except one which was slightly overlong (fig 26).

*I Partial auriculo ventricular block* Five cases of partial auriculo-ventricular block have been examined and are charted in figure 27. One was electrocardiographed during a period of high grade partial block at two different rates in each case. All measurements of electrical systole except the two of one of the cases are within normal limits though varying rather widely. The one case with prolonged Q-T interval during both fast and slow rates with block was a boy of six years with military tuberculosis.

*J Complete auriculo ventricular block* Six cases of complete auriculo-ventricular block showed abnormally long Q-T intervals in all except two and those two were close to the upper edge of normal. However, three of these four cases with overlong Q-T intervals had also bundle branch block and the Q-T intervals were extraordinarily prolonged, with one exception the longest that we have encountered (all over 0.6 second in duration). One of them with varying bundle branch block gave a measurement of the Q-T interval of over 0.7 second, the longest of our entire series and about 0.2 second above

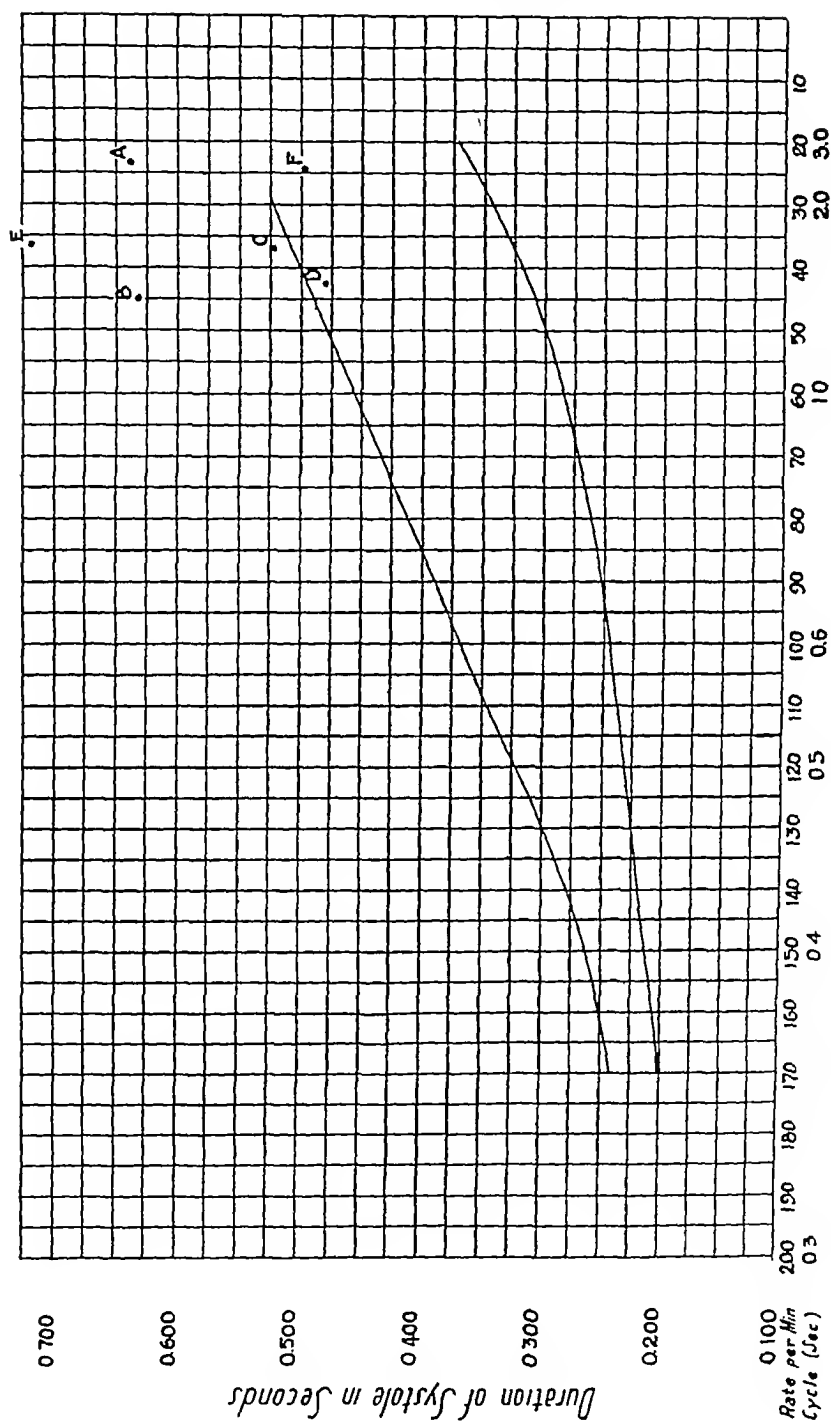


FIG 28 THE Q-T INTERVALS OF SIX PATIENTS WITH COMPLETE AURICULO-VENTRICULAR BLOCK  
Cases A, B and E had also bundle branch block

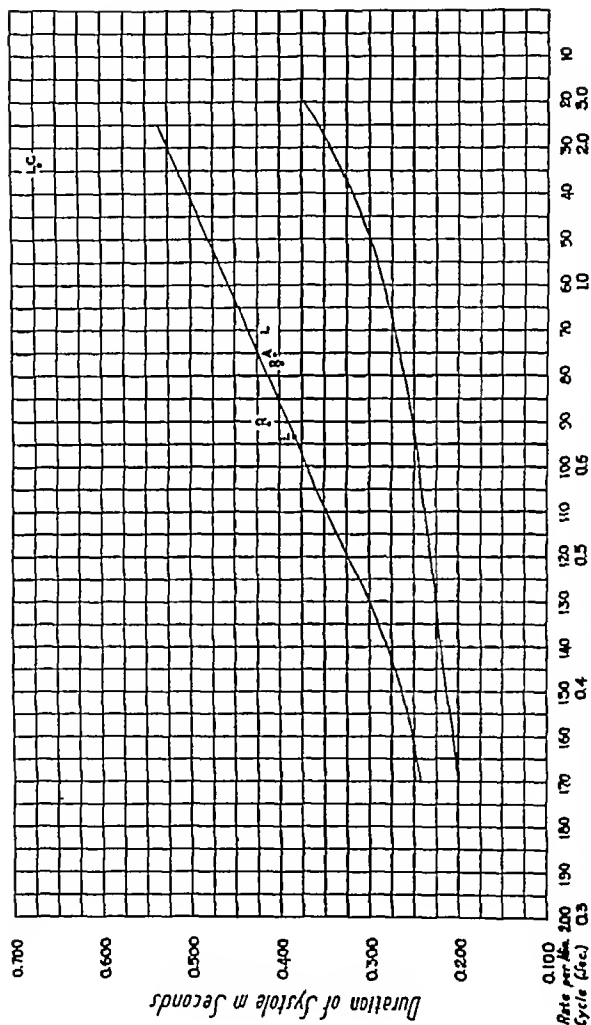


FIG 29 THE Q-T INTERVALS OF SIX PATIENTS WITH BUNDLE BRANCH BLOCK

Cases L, A, L had left bundle branch block, case LC left bundle branch block and complete auriculo-ventricular block, cases R, R right bundle branch block, and case A intraventricular block of lesser grade

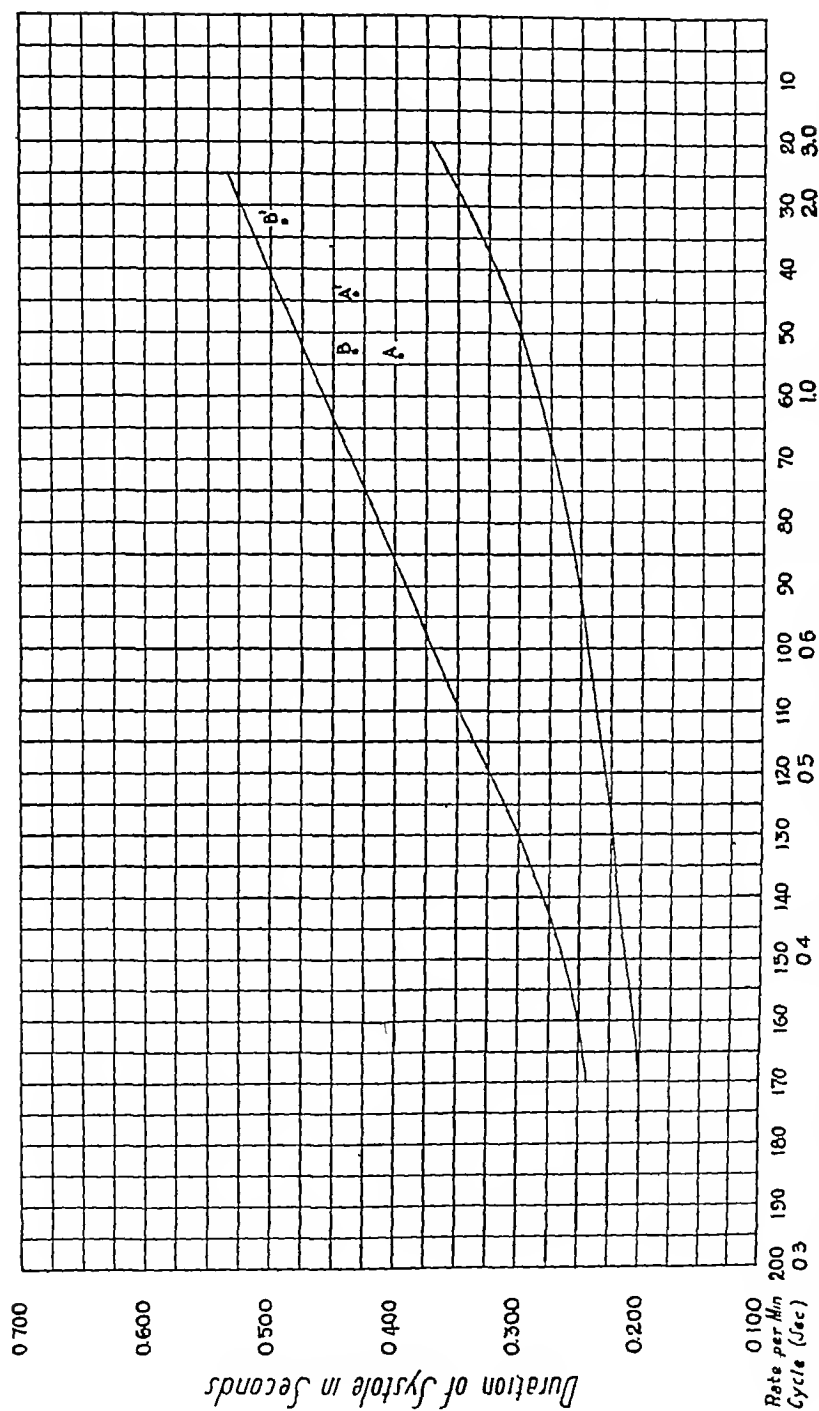


FIG 30 THE Q-T INTERVALS OF TWO CASES DURING ATRIOVENTRICULAR NODAL RHYTHM (A', B') AND DURING NORMAL RHYTHM (A, B)

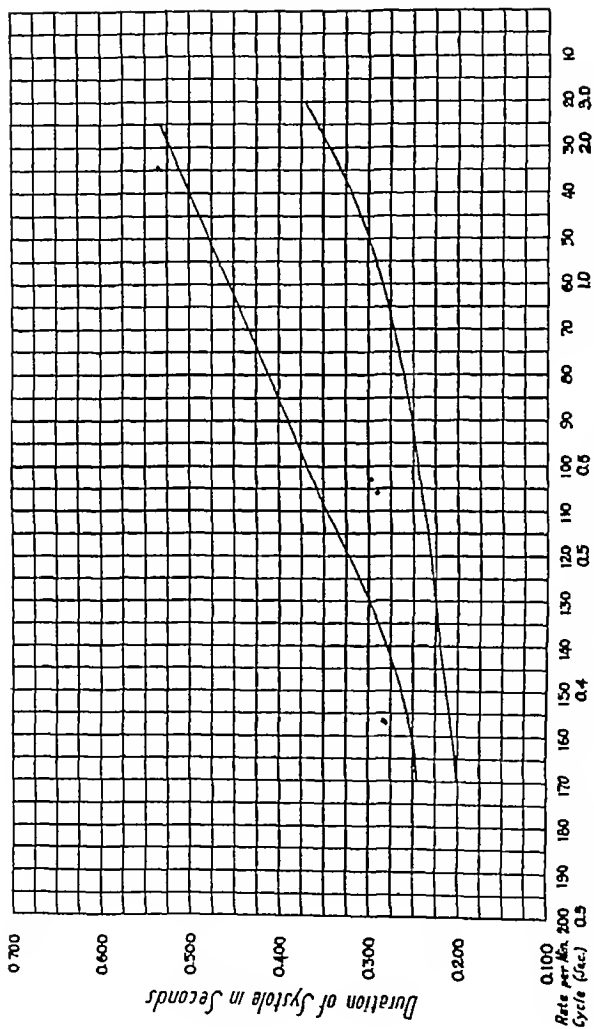


FIG 31 THE Q-T INTERVALS OF FIVE PATIENTS WITH LOW VOLTAGE, ONE OF WHOM HAD ALSO COMPLETE AURICULO-VENTRICULAR BLOCK

the normal upper limits Of the three cases of complete auriculo-ventricular block without bundle branch block only one had a Q-T interval beyond normal limits and that was close to the line (fig 28)

*K Bundle branch block* Six further cases of bundle branch block (in addition to the three already noted under complete auriculo-ventricular block, and the one under aortic stenosis) have been studied Two showed right branch block and in one of these the Q-T interval was just below and in the other just above the outer limit of normal Three cases showed left bundle branch block, the Q-T interval in one of these was just within normal limits, one was on the border line itself, and one was very far beyond normal limits (nearly 0.7 second) In this last case, complete heart block was also present and so that instance should be added to the three already described with extremely long Q-T intervals under the heading of complete heart block The sixth case showed a lesser grade of intraventricular block and its Q-T interval was barely within normal limits However, all six cases were well above the normal average for duration of Q-T interval and near or beyond the upper normal limits This finding is to be expected in view of the slow distribution of stimulus through the ventricles and inefficient onset of ventricular contraction (fig 29)

*L Atrioventricular nodal rhythm* Two cases of the rare atrioventricular nodal rhythm were studied, one also during normal rhythm and the other during auricular fibrillation Both showed normal measurements for the Q-T intervals in each instance (fig 30)

*M Low voltage of electrocardiogram* Finally, five cases showing low voltage by electrocardiogram were studied All showed Q-T intervals within normal limits except one with a very low pulse rate (38) due to complete heart block (fig 31)

#### SUMMARY AND CONCLUSIONS

1 A study is here reported of the measurements of the Q-T interval made by us on carefully selected electrocardiographic plates with the help of the Lucas comparator in 213 individuals, of whom 50 were normal to act as controls (20 men, 15 women and 15 children), and 163 were abnormal subjects to illustrate the effect of various pathological conditions

2. A brief review of the literature concerning the effect of various

factors on the duration of systole has been given, and references to papers listed in a bibliography

3 The interval from the beginning of the Q-R-S complex to the end of the T wave in satisfactory electrocardiograms may be considered as indicative of the duration of electrical systole, and perhaps is a more accurate measurement of actual systolic duration than any other. The more accurately mechanical records of ventricular contraction are obtained, the more closely does the duration of mechanical systole approach that of electrical systole.

4 The prime factor influencing the duration of both mechanical systole and the Q-T interval of the electrocardiogram has been found almost invariably by previous workers to be heart rate. This we have confirmed in our electrocardiographic study. The faster the heart rate the shorter the duration of systole and of the Q-T interval, although at faster heart rates the relative proportion of the heart cycle made up by systole steadily increases, as shown by the slope of the curve in figure 1. Whether the amount of blood entering the heart at various rates is a controlling factor is not clear.

5 As a standard for judging the normal duration of electrical systole we have used a chart on which have been plotted according to heart rate and duration of the Q-T interval (coordinates) first the measurements of our 50 normal cases (fig 1) and then those of 190 normal cases from the literature (fig 2). The outer limits of these 240 normal case measurements have then been joined by curve lines and the resulting figure has been used for judgment as to variation from the normal of our 163 abnormal cases. This method has seemed to us considerably more satisfactory than the application of any formula (no suitable formula has been devised). Also our method has the advantage of rapid graphic illustration. Against the normal curves have been plotted the measurements of the Q-T interval in all of our 163 abnormal cases divided into groups according to the abnormal condition present.

6 For the 240 normal cases the outer limits of normal for the Q-T interval varied from 0.480 to 0.300 second at a heart rate of 50 and heart cycle of 1.2 second to 0.262 to 0.214 second at a heart rate of 150 and heart cycle of 0.4 second. There is thus at slower pulse rates a wide normal variation perhaps due to variations in diastolic filling of the heart. The curve (fig 2) resembles somewhat a comet track

7 In normal individuals, the Q-T interval remained normal with change in position, exercise and digitalization

8 Of all the 163 abnormal cases, only two gave a duration of Q-T interval slightly shorter than normal and those were both cases with hyperthyroidism, one of whom also had auricular fibrillation and the other a well marked tachycardia Both of these cases deviated but little from the normal (0.018 second in one and 0.025 second in the other) No case in the entire series of 163 abnormal individuals gave a duration of systole much shorter than normal

9 A number of the 163 abnormal cases showed Q-T intervals longer than normal Of these the most striking and uniform were four groups, first those cases with bundle branch block (six out of ten, the remaining four being on or close to the upper edge of normal), second those cases with very low blood serum calcium content (both of two cases), third the complexes of ventricular paroxysmal tachycardia (both of two cases) and of ventricular premature beats (all of four cases), and fourth four out of five cases of auricular paroxysmal tachycardia Bundle branch block, ventricular premature beats and ventricular paroxysmal tachycardia are alike in the abnormal and slow distribution of the excitation wave through the entire ventricular musculature The rates for the auricular paroxysmal tachycardia were so fast that we had no normal curves for comparison except by projection which is based on supposition only

10 The slow pulse of complete heart block (seven of nine cases, four of which seven, however, had bundle branch block also and one low voltage), of one case of sinoauricular bradycardia (out of seven), and of one case of partial auriculoventricular block (out of five) was associated with an abnormally long Q-T interval The longest intervals of all were found in four cases of combined bundle branch block and complete heart block and they were 0.718 second (at rate of 36), 0.668 second (at rate of 34), 0.638 second (at rate of 23) and 0.632 second (at rate of 45) These were the only Q-T intervals in the entire series of 213 cases which measured more than 0.6 second long

11 A few other scattered cases showed Q-T intervals slightly longer than normal They were one case (out of five) of uremia, one case (out of nineteen) with a very large heart, one case (out of five) of

auricular premature beats (three others were on or near the upper normal limit line), and one case of auricular fibrillation with a very rapid ventricular rate. Thus there were altogether of the 163 abnormal cases, 22 which showed overlong Q-T intervals and 18 of those showed either bundle branch block, very slow pulse rates, as in complete heart block, very fast pulse rates as in auricular or ventricular paroxysmal tachycardia, ventricular premature beat complexes, or too low a content of calcium in the blood serum.

12 Of pathological conditions, cardiac enlargement, aortic regurgitation, aortic stenosis, mitral stenosis, congenital cardiac defects, hypertension, diabetes mellitus, hyperthyroidism, hypothyroidism, acute infection, congestive failure, auricular fibrillation, atrioventricular nodal rhythm and low voltage in themselves did not affect the duration of the Q-T interval in the series studied here.

13 The measurement of the duration of the Q-T interval of the electrocardiogram is apparently of little or no clinical value.

We take great pleasure in acknowledging the help that has been given to us in the measurements of the Q-T interval by Drs Howard B Sprague, T Duckett Jones, J Francis Kellogg, and Paul V Ledbetter.

#### BIBLIOGRAPHY

- 1 Fridencia, L. S., *Acta Med. Scand.*, 1920, liii, 469 and 489. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken I and II.
- 2 Fridencia, L. S., *Acta Med. Scand.* 1920-1921, liv, 17. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. III. Pathologische Änderungen in der Dauer des Herzventrikel-Elektrokardiogramms.
- 3 Garrod, A. H., *Proc. Roy. Soc. London*, 1870, xviii, 351. On the Relative Duration of the Component Parts of the Radial Sphygmograph Trace in Health.
- 4 Garrod, A. H., *Ibid.*, 1871, xix, 318. On the Mutual Relations of the Apex Cardiograph and the Radial Sphygmograph Trace.
- 5 Garrod, A. H., *Ibid.*, 1875, xxii, 140. On Some Points Connected with the Circulation of the Blood Arrived at from a Study of the Sphygmograph Trace.
- 6 Garrod, A. H., *J. Anat. Physiol.*, 1871, v, 17 and 265. On Cardiograph Tracings from the Human Chest Wall. On the Construction and Use of a Simple Cardio-Sphygmograph.

- 7 Thurston, E, J Anat Physiol, 1876, x, 494 Length of Systole of the Heart as Estimated from Sphygmographic Tracings
- 8 Landois, L, Graphische Untersuchungen über den Herzschlage, Berlin, 1876
- 9 Waller, A D, J Physiol, 1887, viii, 229 A Demonstration on Man of Electromotive Changes Accompanying the Heart's Beat.
- 10 Edgren, J G, Skand Arch f Physiol, 1889, i, 67 Cardiographische und Sphygmographische Studien
- 11 Stockmann, F, Über die zeitliche Verhältnis der Dauer der Systole zur Dauer der Diastole Inaug Diss, Königsberg, 1889
- 12 Kraus, F, Wien klin Wchnschr 1891, iv, 777 Über eine Aenderung im Phasenablauf des arbeitenden Herzens bei Aortenklappen-Insuffizienz
- 13 Lüderitz, C, Ztschr f klin Med, 1892, xx, 374 Versuche über den Ablauf des Blutdruckes bei Aortenstenose
- 14 Einthoven, W, and de Lint, K, Arch f d ges Physiol, 1900, lxxx, 139 Ueber das normale menschliche Elektrokardiogramm und über die capillarelektrometrische Untersuchung einiger Herzkranken
- 15 Zuntz, L, and Schumburg, W A E F, "Studien zu einer Physiologie des Marsches, Berlin, Hirschwald, 1901
- 16 Kraus, F, and Nicolai, G F, Das Elektrokardiogramm des gesunden und kranken Menschen, Leipzig, 1910
- 17 Lipschutz, L, Das Verhalten des Herzens bei Sportlichen Maximalleistungen Inaug Diss, Berlin, 1912
- 18 Hecht, A F, Ergebn d inn Med u Kinderh 1913, xi, 324 Der Mechanismus der Herzaktion im Kindesalter, seine Physiologie und Pathologie
- 19 Samojloff, A, Arch f d ges Physiol, 1910, cxxv, 417 Weitere Beiträge zur Elektrophysiologie des Herzens
- 20 Sternopulo, S, Ztschr f exper Path u Therap, 1909, vii, 467 Das Elektrokardiogramm bei Herzfehlern
- 21 de Heer, J L, Arch f d ges Physiol, 1912, cxlviii, 1 Die Dynamik des Säugetierherzens im Kreislauf in der Norm, bei Aortenstenose und nach Strophantin
- 22 Wiggers, C J, Arch Int Med, 1917, xx, 93 The Electrocardiogram, its Relation to Cardio-dynamic Events
- 23 Einthoven, W, Dunham Lectures, Harvard Medical School, 1924
- 24 Sprague, H B, and White, P D, J Clin Invest., 1925, i, 389 Clinical Observations on the T Wave of the Auricle Appearing in the Human Electrocardiogram.
- 25 Brugsch, T, and Blumenfeldt, E, Berl klin Wchnschr 1919, lvi, 937 Die Leistungszeit des Herzens und ihre klinisch-diagnostische Bedeutung
- 26 Lombard, W P, and Cope, O M, Am. J Physiol, 1919, xlix, 139 Effect of Pulse Rate on the Length of the Systoles and Diastoles of the Normal Human Heart in the Standing Position
- 27 Katz, L N, J Lab and Clin Med, 1921, vi, 291 Factors Modifying the Duration of Ventricular Systole

- 28 Meakins J , Arch Int. Med , 1919, xxiv, 489 Prolongation of the "S-T" Interval of the Ventricular Complex as Shown by the Electrocardiograph
- 29 Hofmann, F B , Ztschr f d ges Exper Med., 1920, xi, 156 Zum Vergleich des Ablaufs des Elektrokardiogramms mit der Stärke der Herzkontraktion.
- 30 Bazett, H C , Heart, 1920, vii, 353 An Analysis of the Time Relations of Electrocardiograms
- 31 Buchanan J A., Arch Int. Med , 1921, xxviii, 484 A Study of the S T Interval in 1028 Electrocardiograms
- 32 Miki, Y, Ztschr f d ges Exper Med , 1922, xxvii, 323 Experimentelle und Klinische Untersuchungen über die Dauer des K Ekg (Kammer Elektrokardiogramms)
- 33 Tigerstedt, R., Die Physiologie des Kreislaufs, 2 Aufl , Berlin, Leipzig, 1921
- 34 Carter, E P , and Andrus, E C , Trans. Am. Soc. Clin Invest., 1922, vol. 15 Q-T Interval in Human Electrocardiogram in Absence of Cardiac Disease.
- 35 Fenn, G K , Arch Int. Med , 1922, xlix, 441 Studies in the Variation in the Length of the Q R S-T Interval
- 36 Katz, L N , and Feil, H S , Arch Int Med., 1923, xxxii, 672 Clinical Observations on the Dynamics of Ventricular Systole I Auricular Fibrillation
- 37 Feil, H S , and Katz, L N , Arch Int. Med , 1924, xxxiii, 321 Clinical Observations on the Dynamics of Ventricular Systole II Hypertension
- 38 Katz, L N , and Feil, H S , Heart, 1921, xii, 171 Clinical Observations on the Dynamics of Ventricular Systole III Aortic Stenosis and Aortic Insufficiency
- 39 Bartos, E , and Burstein, J J Lab and Clin Med., 1924, ix, 217 Can Variations in Ventricular Systole Be Determined from Electrocardiogram Deflections?
- 40 Frey, W , Ztschr f d ges Exper Med , 1924, xlii, 614. Die mechanische Latenzzeit des Herzmuskels I Mitteilung Besitz der Herzmuskel überhaupt eine mechanische Latenzzeit?
- 41 Lombard, W P , and Cope, O M , Am J Physiol., 1926, lxxvii, 263 The Duration of the Systole of the Left Ventricle of Man
- 42 Seham, M , Abt's Pediatrics, 1924, vol iv, 295 Electrocardiography in Children



# THE CALORIGENETIC ACTION OF THYROXIN AT DIFFERENT LEVELS OF BASAL METABOLISM IN MYXEDEMA

BY WILLARD OWEN THOMPSON<sup>1</sup> PHEBE K. THOMPSON<sup>2</sup> ALLEN G. BRAILEY, AND ARCHIBALD C. COHEN

*(From the Thyroid Clinic and the Metabolism Laboratory of the Massachusetts General Hospital)*

(Received for publication March 22 1929)

## INTRODUCTION

In the course of a study of the change in plasma volume accompanying treatment with desiccated thyroid in myxedema (1), it was noted that after the basal metabolic rate had been restored to normal, further increases in thyroid dosage up to four times the maintenance requirement produced proportionately much smaller increases in basal metabolism than did the maintenance dose. The effect at a normal level, in fact, appeared to be of about the same magnitude as that produced by giving thyroid to a normal individual.

It seemed desirable to confirm these observations, using thyroxin intravenously instead of desiccated thyroid by mouth, in order to eliminate the possibility of non absorption of the latter from the gastro intestinal tract.

## METHOD

Accordingly, two patients with untreated typical myxedema were each given 10 mgm. of thyroxin intravenously, and the influence on heat production and on clinical symptoms were compared with those following the same dose given to each patient at a later period when the basal metabolism was maintained at a normal level by desiccated thyroid (figs 1 to 6).

Both patients were kept in the hospital on routine diet throughout the "thyroxin curve" periods, with the exception of the second patient

<sup>1</sup> Fellow of the National Research Council Research Fellow in Medicine, Harvard Medical School and Massachusetts General Hospital

<sup>2</sup> Research Fellow in Medicine Massachusetts General Hospital

who was not in the hospital during the time covered by the latter two-thirds of the first curve

It is very important to keep the patient for a long time on a given dose of desiccated thyroid before drawing the conclusion that a metabolic level is established on that dose. The first patient was kept for four months on three grains of desiccated thyroid daily before the second injection of thyroxin. The second patient was kept for nearly two months on three grains, and then for four months on one and one-half grains of desiccated thyroid daily before the second injection of thyroxin, and on three grains daily for nine months before the third injection. Moreover, after a level had been established on these doses at home, a change occurred after return to the hospital the metabolism determinations were usually lower, evidently due to the elimination of a rather long journey to the hospital before each test.

In order to keep conditions constant throughout the experiment, *the daily doses of desiccated thyroid were continued following the second and third injections of thyroxin*

Basal metabolic rates were determined with the Roth-Benedict apparatus, using Aub-DuBois standards

We used Squibb's thyroxin and Armour's desiccated thyroid

#### DATA

The course of the basal metabolic rate, of the pulse and of the weight in each instance, is depicted in figures 1 and 2. The effect of each injection on the basal metabolism is shown in detail in figures 3 and 4. The temperature reactions after each injection are shown in figures 5 and 6.

The general clinical course of the toxic effects of an injection of thyroxin has been described by Boothby, Sandiford, Sandiford and Slosse (2), but detailed descriptions of the onset and duration of both toxic and beneficial effects in their relation to metabolism, have not been reported. For this reason, the clinical histories of these two patients are given in detail.

*Case 1* (figs 1, 3 and 5) Lab No 4671 Mrs E G Age 48 A case of untreated typical myxedema. On entering the hospital May 7th, 1927, her face was puffy, and she had swelling of the hands and legs which felt numb and clumsy. She had gained 60 pounds in the last four years. Her tongue was thick and her

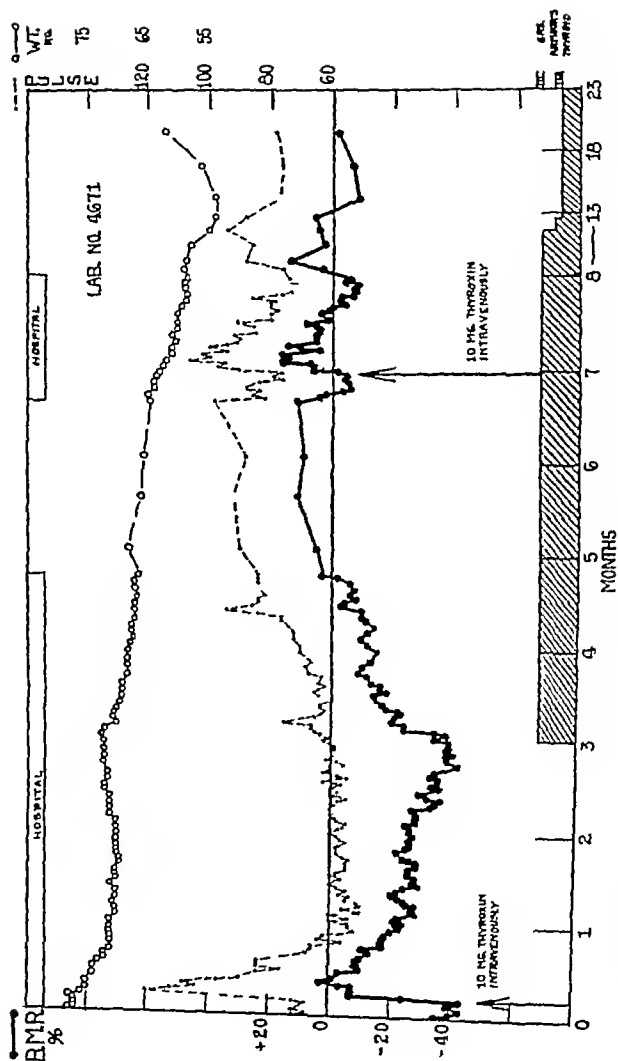


FIG 1 CASE 1 HEIGHT 170 CM THE EFFECT ON BASAL METABOLIC RATE, PULSE AND WEIGHT OF THE INTRAVENOUS INJECTION OF 10 MCGM OF THYROXIN, IN A PATIENT WITH MYXEDEMA, AT DIFFERENT LEVELS OF BASAL METABOLISM

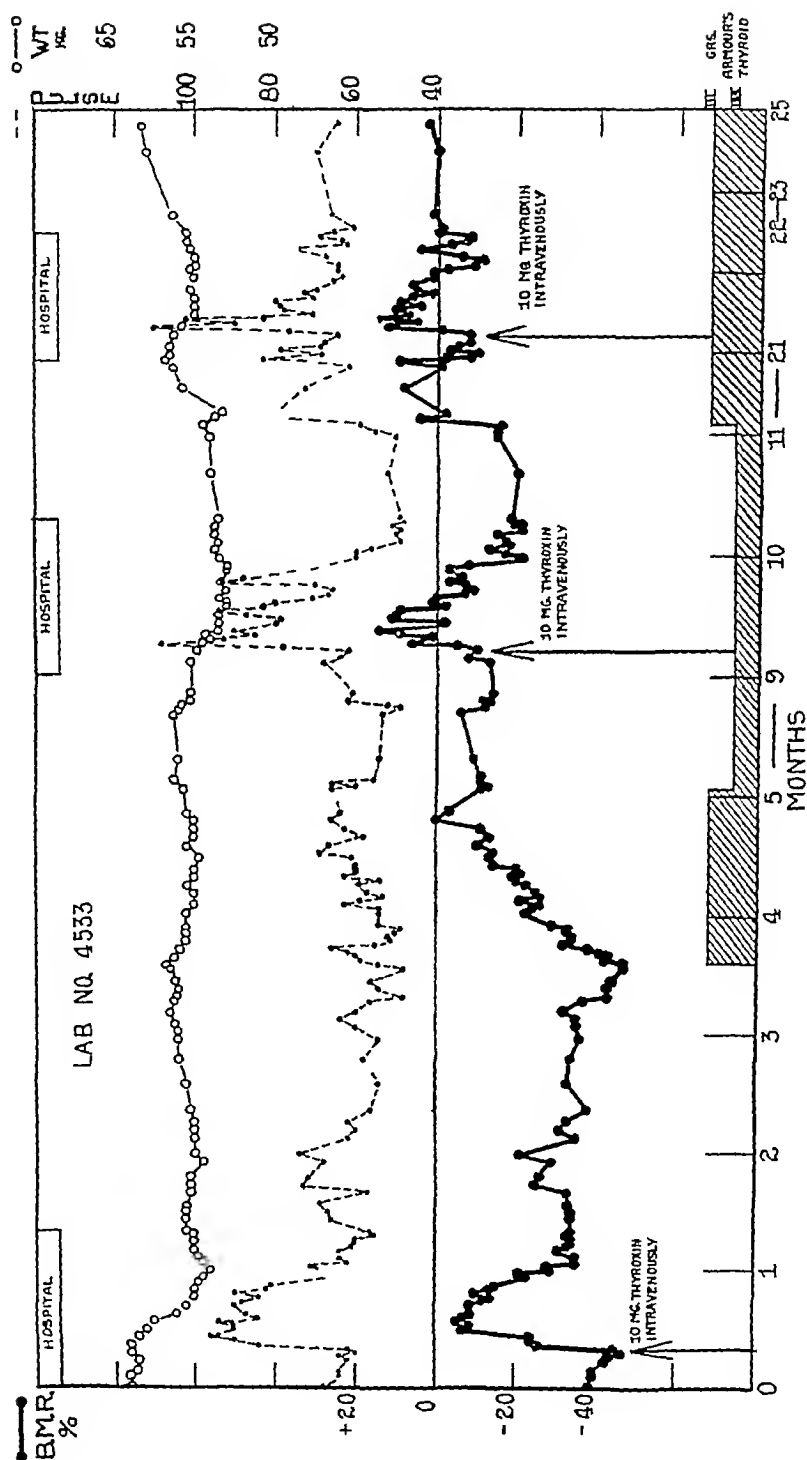


FIG 2 CASE 2 HEIGHT 160 CM ALSO SHOWING THE EFFECT ON BASAL METABOLIC RATE, PULSE AND WEIGHT OF THE INTRAVENOUS INJECTION OF 10 MG OF THYROXIN, IN A PATIENT WITH MYXEDEMA, AT DIFFERENT LEVELS OF BASAL METABOLISM (MINUS 45 PER CENT, MINUS 13 PER CENT AND MINUS 6 PER CENT)

Note that in both figure 1 and figure 2, the daily dose of desiccated thyroid which had been used to establish the higher levels of basal metabolism before the injections of thyroxin, was continued throughout the period that the thyroxin had any effect

hair very dry and brittle. Her skin was dry and scaly and showed marked pigmentation on the face and arms. Her voice was hoarse and her speech extremely slow and deliberate. She felt sleepy, weak and cold all the time, and had so dyspnoea and palpitation on exertion. She never perspired. Her appetite was poor and she was constipated. She was markedly slowed mentally, her memory was very poor, and she was somewhat deaf. Her catamenia had been absent six years. Her basal metabolic rate was minus 40 per cent.

On May 14th at 3 p.m. she was given 10 milligrams of thyroxin intravenously. At 6 p.m. her skin felt warmer and her pulse had risen slightly. She had no palpitation during the night. Next morning she appeared brighter and taller and faster. The edema of her eyelids had noticeably diminished. There was slight pain on moderate pressure of muscles of the right neck, upper arm and the lower half of both legs. The following day she had marked palpitation and as if she were burning up. She was nauseated and vomited three times. She had a slight watery nasal discharge. On the 17th the edema of her eyelids was practically all gone. Her mouth and lips were parched. She was thirsty and was passing more urine than usual. There was no increase in muscle soreness. The next day she noticed that she felt less drowsy and that the skin on her face was softer, although it was still rough and dry on her hands. Her tongue felt as though it had "more room" in her mouth. On the 19th muscle soreness had increased somewhat. On the 20th it was noted that the skin of her hands was definitely smoother and softer. Her heart was much quieter. Her facial expression had completely changed, due to the disappearance of edema. Her basal metabolism had reached its highest level of plus 3 per cent. By June 25th her skin was peeling all over her body. She felt brighter and stronger and moved about much more quickly. The muscle soreness was not as marked. The pigmentation of her skin was beginning to fade. Her hair was starting to fall out. By the 30th the desquamation of her skin was very marked, particularly on her legs. Although still edematous at the ankles, her legs felt much less heavy and numb. She did not mind the cold and her appetite was improving. Her voice was much less hoarse and she talked much more quickly than on October 1. June 3rd the peeling of the skin was less marked and her hands did not feel as stiff. Her ankles were still swollen slightly, but there was no edema of the face. On June 11th she stated that her hearing was better and that she could think more quickly than before the injection of thyroxin. She could also move her fingers and toes more easily. June 15th she was still improving although her basal metabolism had fallen to minus 28 per cent. June 18th edema of the eyelids began to recur and she began to feel as if she would enjoy a nap after lunch. She had had no ankle swelling for about a week. July 2nd she was still feeling better and was able to walk around the hospital yard six times without resting—the first time she had accomplished such a feat. July 4th her voice was slightly hoarse and she talked a trifle more slowly. The pigmentation of her skin had faded still more. July 12th the skin had stopped peeling on her face but was still peeling marked

on her palms She found it harder to keep balanced when walking July 20th she felt more drowsy July 29th her lower eyelids were markedly swollen on arising The thick heavy skin on the soles of her feet had begun to peel Her basal metabolism was minus 33 per cent August 8th she had some numbness of the fingers of her left hand Her skin was slightly dry and it had just finished peeling on her hands Her hair was not falling out She was somewhat slowed up and her walking was a little unsteady Her tongue was gradually becoming thick again Marked edema of the eyelids was present on arising, but there was no noticeable increase in edema elsewhere She was not cold and said that she felt much stronger and brighter than when she entered the hospital Her basal metabolism had reached its pre-injection level of minus 40 per cent

On August 9th she was started on 3 grains of Armour's desiccated thyroid daily Within two days she felt brighter and less sleepy By August 16th the puffiness of her eyelids had diminished considerably and by August 29th it was gone She felt tired however By September 3rd the skin on her feet had stopped peeling She had slight muscle and joint pains Her hair was falling out a little but new hair was growing in October 1st she was discharged from the hospital with a normal basal metabolism Her skin was smooth except for some peeling on her arms and hands There was no edema of her eyelids Her feet felt more supple and she was stronger and brighter than one month previously The pigmentation on her arms and face had faded markedly While home, she became more nervous, tired easily and appeared a little "washed out" October 23rd she had her first normal catamenia for seven years She was readmitted to the hospital November 29th In a few days she felt more rested Her level of basal metabolism was minus 4 per cent

She was given 10 mgm of thyroxin intravenously at 12 p m December 8th Desiccated thyroid (3 grains daily) was continued December 9th she noted no change except that she felt warmer, her heart pounded somewhat and the muscles of her left arm were slightly tender That night she had a slight feeling of suffocation and had "catching pains" in her left axilla for about 15 minutes December 10th she was a little nervous and "shaky" By the 11th she was still feeling warmer and perspiring more Her pulse became rapid only on exertion She had anorexia and slight nausea but no vomiting On the 12th she was very thirsty and was unsteady on her feet December 13th, when in bed, she felt well, when walking about she got "shaky" She had attacks of marked perspiration and her mouth felt dry and parched December 14th she was tremulous, had intermittent muscle pains, and felt hot and fidgety On the 15th she felt much better Her appetite was improving December 16th, although still somewhat fidgety, her muscle pains had disappeared She noticed a marked increase in salivation since receiving thyroxin She had practically no palpitation On the 18th she still felt unusually warm. The pigmentation of her skin had almost completely faded December 20th she had no complaints She felt better than she did on entry in that she was more rested Her nervousness had practically disappeared On the 29th she was clinically the same Her skin was still

slightly more warm and moist than normal, although this had decreased noticeably within the last week. Her hair had begun to fall out about one week previously. By January 1st, 1928 her basal metabolism had returned to its pre-injection level. On the 7th she was discharged from the hospital, still taking 3 grains of desiccated thyroid daily. After she went home, as she was tiring easily and was nervous and restless at night, perspired easily and had some precordial pain and palpitation, her dose of thyroid was reduced to 2 grains daily on April 28th and to  $1\frac{1}{2}$  grains daily on May 25th. On this smaller dose she gradually became stronger. When last seen December 21st, 1928, she was well, had no palpitation, was not nervous, did not tire easily and weighed a little more than when taking 3 grains of thyroid daily. There were no signs or symptoms of myxedema. Her basal metabolism was minus 2 per cent.

*Case 2* (figs. 2, 4 and 6) Lah No 4533 Mrs. M B Age 30 On entering the hospital February 19th, 1927, she was a classical case of untreated myxedema. She had been steadily gaining weight and had had no catamenia for five years. Her tongue, face, hands, abdomen and legs were swollen. Her hair was coarse and dry. Her skin was very dry and scaling and she never perspired. Her speech was slow. She was always cold and drowsy. Her appetite was poor, she was nauseated in the mornings and was very constipated. Her mental processes had become very slow and her memory poor. She was ataxic and had frequent headaches. The level of her basal metabolism was minus 45 per cent.

On March 3rd, at 3 p.m. she was given 10 mgm of thyroxin intravenously. At midnight she passed an unusually large amount of urine. All the next day she had a severe headache and marked nausea and anorexia, with increased thirst. She felt warm and objectively her face felt very hot. There was a noticeable diminution in the edema of her eyelids. She had slight pains all over her body and by evening her arm and leg muscles were sore on moderate pressure. March 5th her headache was less severe. She felt very hot—her temperature was  $104^{\circ}\text{F}$ . In the afternoon all her tissues all over her body were extremely sore. She sweated profusely all night. Next day her muscle soreness was much less, although still marked in the leg muscles. She was nervous and tremulous. The edema of her face had markedly diminished. March 10th her basal metabolism had reached its highest level of minus 6 per cent. For the first time in several days she was able to eat a moderate amount. She talked faster and louder and felt much brighter. She was still thirsty and passing an increased amount of urine. On the 12th the swelling had disappeared from her face and none was noticeable elsewhere in the body. She had no headache. She shook violently on trying to stand up. She had had no nausea and vomiting for two days and her muscle soreness had subsided. She looked much brighter. The skin on her hands was more moist and smooth and the skin on her face and feet had just begun to peel. March 14th she felt much improved and could walk better than for a week. Her appetite was fair. By the 20th she was "feeling good," but she could not eat. On the 23rd her skin was still dry on her trunk, although definitely softer on her

on her palms She found it harder to keep balanced when walking July 20th she felt more drowsy July 29th her lower eyelids were markedly swollen on arising The thick heavy skin on the soles of her feet had begun to peel Her basal metabolism was minus 33 per cent August 8th she had some numbness of the fingers of her left hand Her skin was slightly dry and it had just finished peeling on her hands Her hair was not falling out She was somewhat slowed up and her walking was a little unsteady Her tongue was gradually becoming thick again Marked edema of the eyelids was present on arising, but there was no noticeable increase in edema elsewhere She was not cold and said that she felt much stronger and brighter than when she entered the hospital Her basal metabolism had reached its pre-injection level of minus 40 per cent

On August 9th she was started on 3 grains of Armour's desiccated thyroid daily Within two days she felt brighter and less sleepy By August 16th the puffiness of her eyelids had diminished considerably and by August 29th it was gone She felt tired however By September 3rd the skin on her feet had stopped peeling She had slight muscle and joint pains Her hair was falling out a little but new hair was growing in October 1st she was discharged from the hospital with a normal basal metabolism Her skin was smooth except for some peeling on her arms and hands There was no edema of her eyelids Her feet felt more supple and she was stronger and brighter than one month previously The pigmentation on her arms and face had faded markedly While home, she became more nervous, tired easily and appeared a little "washed out" October 23rd she had her first normal catamenia for seven years She was readmitted to the hospital November 29th In a few days she felt more rested Her level of basal metabolism was minus 4 per cent

She was given 10 mgm of thyroxin intravenously at 12 p.m December 8th Desiccated thyroid (3 grains daily) was continued December 9th she noted no change except that she felt warmer, her heart pounded somewhat and the muscles of her left arm were slightly tender That night she had a slight feeling of suffocation and had "catching pains" in her left axilla for about 15 minutes December 10th she was a little nervous and "shaky" By the 11th she was still feeling warmer and perspiring more Her pulse became rapid only on exertion She had anorexia and slight nausea but no vomiting On the 12th she was very thirsty and was unsteady on her feet December 13th, when in bed, she felt well, when walking about she got "shaky" She had attacks of marked perspiration and her mouth felt dry and parched December 14th she was tremulous, had intermittent muscle pains, and felt hot and fidgety On the 15th she felt much better Her appetite was improving December 16th, although still somewhat fidgety, her muscle pains had disappeared She noticed a marked increase in salivation since receiving thyroxin She had practically no palpitation On the 18th she still felt unusually warm. The pigmentation of her skin had almost completely faded December 20th she had no complaints She felt better than she did on entry in that she was more rested Her nervousness had practically disappeared On the 29th she was clinically the same Her skin was still

knee jerks, was probably unrelated to her myxedema. There was no essential change in her basal metabolism.

November 28th, she was given 10 mgm. of thyroxin intravenously at 8 p.m. Desiccated thyroid, grains  $1\frac{1}{2}$  daily, was continued. During the night her head began to ache. Next morning her headache was gone, but she felt more nervous and restless. In the evening she vomited. On the 30th she felt exceedingly warm. Her skin was hot although not moist, and her temperature was 101.5°F. She had a headache and was thirsty. She was not passing more urine. December 1st she had no headache, nausea or fever, but she felt weaker and more nervous. On the 2nd her nervousness had decreased. On the 3rd she felt "fine" and continued thus, except for a transient attack of aches and pains on December 15th. Her basal metabolism reached its pre-injection level of minus 13 per cent about December 21st. On the 31st she was discharged from the hospital, with a basal metabolism of minus 18 per cent. She felt much brighter and livelier than on admission and had no ataxia. One and one-half grains of thyroid was continued at home. On this dose she felt well until February 1928, when she had begun to be doxy and drowsy again. Thyroid was increased to 3 grains daily. On this dose her metabolism rose to normal, she became more nervous and tired more easily. Her appetite was poor. She had no palpitation. She reentered the hospital November 17th. There were no signs or symptoms of myxedema and her basal metabolic level was minus 6 per cent.

She was given 10 mgm. of thyroxin intravenously at 6 p.m., November 26th. At about 10 o'clock that night a dull headache which she had had for several days, became much worse, and she became nauseated. Next morning there was some tenderness of the leg and arm muscles. This became more pronounced towards evening. She was very nervous, had a tremor, was unusually thirsty and perspired a great deal. That night she had severe pain about the umbilicus and vomited four times. On the 28th her nausea and headache were less marked, and her skin was not so warm. Her temperature was 100°F. Her muscles were still sore. On the 29th she was much less nervous and she had no tremor or nausea. Muscle tenderness was much less marked. Her skin temperature was about normal. November 30th she had some palpitation and nausea in the morning, and she felt nervous and jumpy. In the afternoon her heart was quieter. Her skin felt warm and her leg muscles were still quite tender. She was drinking more water and passing more urine. December 1st, her appetite, which had been poor since injection, improved. On the 2nd, muscle tenderness was confined to the legs, which were moderately tender. She had no cardiac symptoms. December 5th she was feeling well. The muscles of her right calf were still slightly tender. By the 7th muscle tenderness had completely disappeared. From then on, her progress was uneventful, except for a mild upper respiratory infection about December 17th. Her basal metabolism reached its pre-injection level of minus 6 per cent about December 19th. She was discharged on 3 grains of thyroid daily, on the 21st. March 11th, 1929, when last seen, she was well.

There were no signs or symptoms of myxedema and her basal metabolism was plus 2 per cent

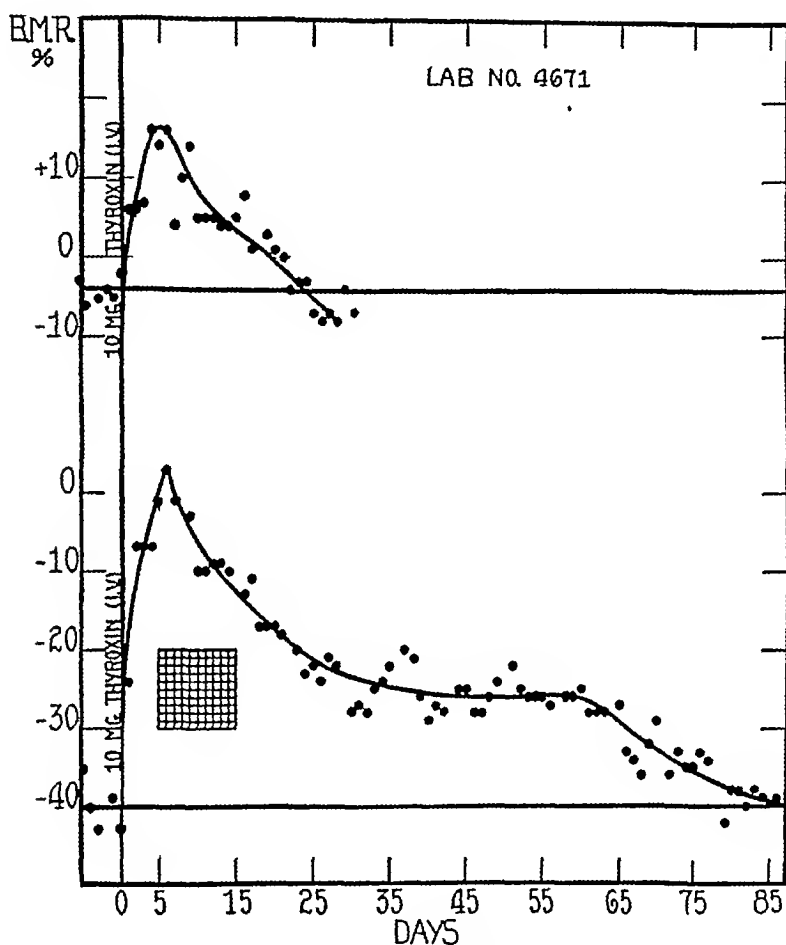


FIG 3 CASE 1 SHOWING IN DETAIL THE MARKED DIFFERENCES IN THE MAGNITUDE AND DURATION OF THE INCREASE IN BASAL METABOLISM FOLLOWING THE INTRAVENOUS INJECTION OF 10 MG. OF THYROXIN, AT DIFFERENT METABOLIC LEVELS (MINUS 40 PER CENT AND MINUS 4 PER CENT)

*Comparison of effect on basal metabolism of the intravenous injection of 10 mgm of thyroxine when the patients were myxedematous and when they were normal*

It may be noted from figures 3 and 4 that, when the patients were myxedematous, the increase in basal metabolism following the intra-

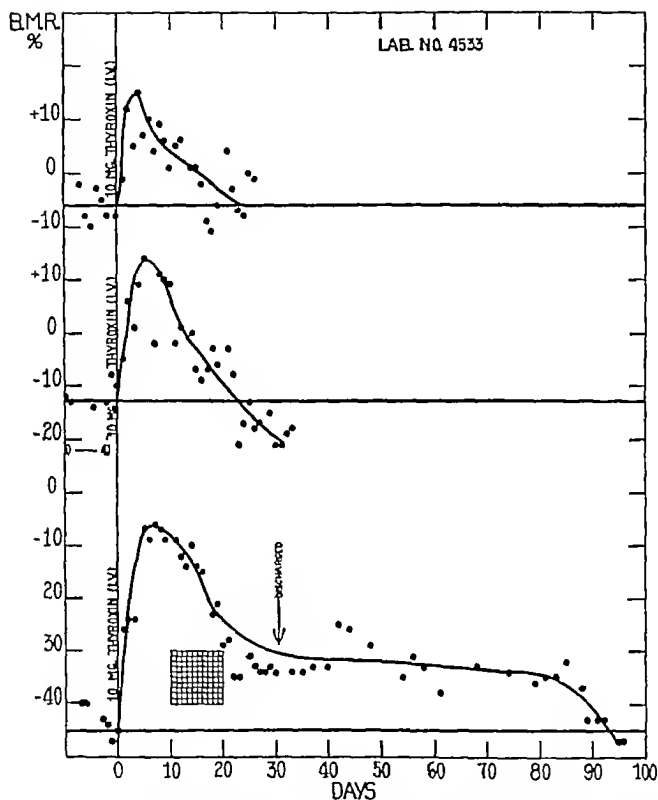


FIG. 4 CASE 2 ALSO SHOWING IN DETAIL THE MARKED DIFFERENCES IN THE MAGNITUDE AND DURATION OF THE INCREASE IN BASAL METABOLISM FOLLOWING THE INTRAVENOUS INJECTION OF 10 MG. OF THYRAXIN, AT DIFFERENT METABOLIC LEVELS (MINUS 45 PER CENT, MINUS 13 PER CENT AND MINUS 6 PER CENT)

venous injection of 10 mgm of thyroxin was of much greater magnitude and of much longer duration than when the patients were normal. These differences are summarized in table 1.

The total excess heat produced by each injection of 10 milligrams of thyroxin was estimated as follows:

The daily metabolic rates were plotted on graph paper ruled in small squares (sample insert in each figure), each small square representing a change of 1 per cent in the standard normal basal calories.

TABLE 1

*Summary of effect on basal metabolism produced by the intravenous injection of 10 mgm of thyroxin at different metabolic levels in myxedema*

| Case number | B M.R. before injection | Surface area before injection | Number of squares | Total excess calories | Excess calories per square meter body surface | Per cent rise in B M.R. per milligram injected | Per cent rise in B M.R. per square meter body surface per milligram injected | Time required for B M.R. to rise to maximum | Total length of time B M.R. was affected | Time required for B M.R. to drop from maximum to pre-injection level |
|-------------|-------------------------|-------------------------------|-------------------|-----------------------|---|--|--|---|--|--|
|             | per cent                | sq m                          |                   |                       |   |  |  | days  | days                                     | days   |
| 1           | -40                     | 1.89                          | 1,375             | 22,455                | 11,880  | 4.3  | 2.3  | 6   | 86                                       | 80   |
|             | -4                      | 1.74                          | 221               | 3,320                 | 1,910   | 2.0  | 1.2  | 4   | 24                                       | 18   |
| 2           | -45                     | 1.65                          | 1,458             | 21,075                | 12,770  | 3.9  | 2.4  | 5   | 93                                       | 85   |
|             | -13                     | 1.57                          | 303               | 4,165                 | 2,655   | 2.7  | 1.7  | 5   | 23                                       | 18   |
|             | -6                      | 1.60                          | 201               | 2,815                 | 1,760   | 2.1  | 1.3  | 4   | 23                                       | 19   |

per square meter per 24 hours. A curve was drawn through the points thus plotted, to denote the actual course of the metabolism. The base line was the level of the metabolism before the injection of thyroxin. The number of squares contained in the area bounded by the base line and the curve was counted. The difference in the number of squares in the graphs at different metabolic levels gives a fairly close estimate of the difference in the magnitude of the response at each level, but to bring each response to terms of total excess heat production, the normal basal metabolic rate (Aub-DuBois standards) in calories for 24 hours was multiplied by the number of squares and the result divided by one hundred.

*Example**Case 1*

Basal metabolic rate before injection, minus 40 per cent

Standard normal calories per square meter per hour = 36.0

Body surface before injection = 1.89 square meters

If metabolic rate were standard normal before injection, total basal calories for 24 hours would be  $36.0 \times 24 \times 1.89$

Number of squares in graph = 1375

Total excess calories produced by 10 mgm. of thyroxin =  $\frac{36.0 \times 24 \times 1.89 \times 1375}{100}$

= 22,455

Total excess calories per square meter produced by 10 mgm. of thyroxin =  $\frac{36.0 \times 24 \times 1375}{100} = 11,880$

The method used is rough, but is sufficiently accurate, considering the number of other variables which enter into the situation, to show the marked differences in reaction which occur at different levels of basal metabolism.<sup>3</sup>

When the patients were myxedematous with basal metabolic rates of minus 40 and minus 45 per cent, the total excess heat produced by the injection of 10 mgm. of thyroxin was  $\frac{22,455}{3,320} = 6.8$  times and

$\frac{21,075}{2,815} = 7.5$  times as great respectively as when they were normal

with rates of minus 4 and minus 6 per cent

It is of interest that the ascending portions of the curves occupied about the same length of time (4 to 6 days) at both the normal and the myxedematous levels, being possibly a little shorter at the normal level

The rate of increase in heat production was greatest immediately following injection, particularly at the myxedematous level. Thus in case 1 it had risen 16 out of 43 points within 18 hours after injection and 33 points within 42 hours. It then remained at a level for another 48 hours before starting to rise to its maximum, which was reached 138 hours after injection. In case 2 it had risen 20 out of 39 points

<sup>3</sup> In each calculation the figure used for surface area is that of the patient just before injection. The ensuing changes in area are not taken into account, because the influence on final results is so slight.

within 18 hours after injection, and then remained at about the same level for another 48 hours before starting to rise to its maximum, which was reached 114 hours after injection

The descending portions of the curves occupied a much longer time when the injection was given at the myxedematous levels (80 to 85 days) than when it was given at the normal levels (18 to 19 days) At the normal levels, the initial part of the fall was perhaps a little steeper than the last part The long plateau, at a level a little above the base line, which was an outstanding feature of the descending portion of the curve when the injection was given at a myxedematous level, was missing

*Comparison of effect on clinical condition of the intravenous injection of 10 mgm of thyroxin when the patients were myxedematous and when they were normal*

As the clinical histories show, there were two outstanding differences

1 The symptoms attributable to intoxication, viz, vomiting, anorexia, fever, headache, palpitation, increased thirst, tremor and muscle tenderness, were all more marked when the patients were myxedematous than when they were normal

2 The clinical improvement at the myxedematous level was striking at the normal level, the only effect was a toxic one

It is of interest that the degree of muscle tenderness and the increase in temperature following injection appear to vary directly with one another Thus, in the first patient, there was only slight muscle tenderness even at the myxedematous level, and the temperature was only slightly elevated (fig 5) at the myxedematous and not at all at the normal level In the second patient, however, the muscle tenderness was very severe at the myxedematous level and the temperature was correspondingly high, viz, 104 degrees Fahrenheit (fig 6) at the normal level both were less affected, but nevertheless were as marked as in the first patient at the myxedematous level These observations suggest that the muscle tenderness and increase in body temperature following the injection of thyroxin, may be due to the same cause, i e, destruction of tissue

A striking feature was the rapidity with which the clinical reaction

appeared following the injections. This was shown particularly when the injection was given at the myxedematous level. Thus, in case 1,

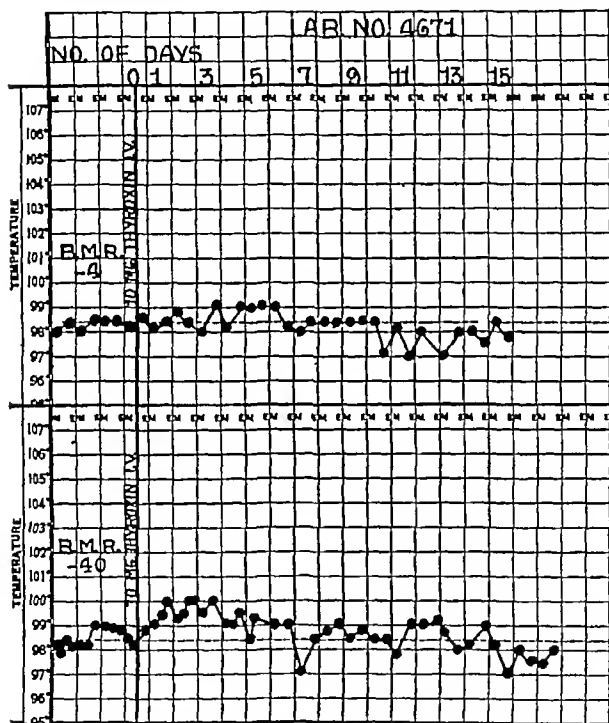


FIG 5 CASE 1 THE EFFECT ON BODY TEMPERATURE OF THE INTRAVENOUS INJECTION OF 10 MG. OF THYRONIN AT DIFFERENT METABOLIC LEVELS (MINUS 40 PER CENT AND MINUS 4 PER CENT)

as evidenced by increased pulse rate and temperature, the reaction had begun 3 hours after injection. In case 2, where the patient was not followed closely immediately afterward, all that was noted was

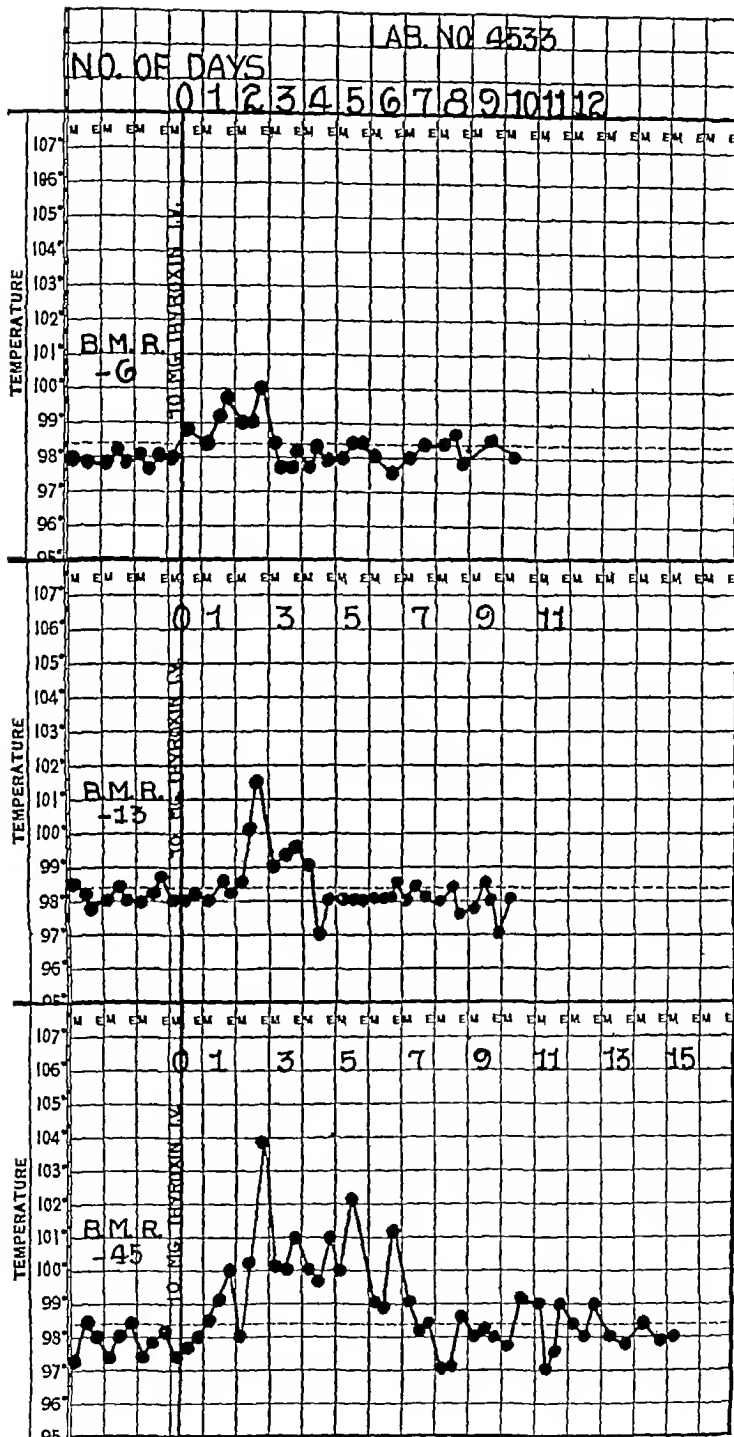


FIG 6 CASE 2 ALSO SHOWING THE EFFECT ON BODY TEMPERATURE OF THE INTRAVENOUS INJECTION OF 10 MG. OF THYROXIN AT DIFFERENT METABOLIC LEVELS (MINUS 45 PER CENT, MINUS 13 PER CENT AND MINUS 6 PER CENT)

TABLE 2

*Comparison of clinical condition of patients before the injection of 10 mgm of thyroxin and after its effect on basal metabolism had worn off (myxedematous level)*

|                        | Case 1  |   | Case 2  |   |
|------------------------|---|---|---|---|
|                        | Before injection of 10 mgm. thyroxin. B.M.R. -40 per cent | 86 days after injection of 10 mgm. thyroxin. B.M.R. -40 per cent  | Before injection of 10 mgm. thyroxin. B.M.R. -45 per cent | 93 days after injection of 10 mgm. thyroxin. B.M.R. -45 per cent              |
| Weight                 | 78 kgm  | 72 kgm  | 63 kgm.   | 58 kgm  |
| Pulse                  | 68  | 62  | 62  | 54  |
| Edema                  | Face, legs hands and abdomen swollen                      | Eyelids swollen No noticeable edema elsewhere Skin on hands loose | Face, legs, hands and abdomen swollen                     | Eyelids slightly swollen. No edema of legs or hands. Abdomen slightly swollen |
| Skin                   | Very dry and scaly Marked pigmentation of face and arms   | Moderately dry Pigmentation markedly faded                        | Very dry and scaly  | Slightly dry  |
| Hair                   | Very dry and brittle                                      | Not dry   | Very dry and coarse                                       | Not dry   |
| Tongue                 | Large and thick   | Slightly thick  | Large and thick   | Normal  |
| Voice                  | Hoarse  | Slightly hoarse   | Hoarse  | Slightly hoarse   |
| Gait                   | Markedly unsteady   | Slightly unsteady   | Markedly ataxic   | Spells of ataxia  |
| Slowness               |   |   |   |   |
| Of movement            | ++++  | +   | +++   | +   |
| Of speech              | +++   | +   | ++  | +   |
| Of thought             | ++  | +   | +++   | +   |
| Hearing                | Poor  | Good  | --  | --  |
| Memory                 | Very poor   | Fair  | Very poor   | Fair  |
| Sensitivity to cold    | +++   | 0   | ++++  | ++  |
| Numness of extremities | ++  | +   | --  | --  |
| Drowsiness             | ++++  | ++  | ++++  | ++  |
| Weakness               | +++   | +   | +++   | +   |
| Headaches              | 0   | 0   | +   | +   |
| Appetite               | Poor  | Fair  | Poor  | Rather poor   |
| Nausea                 | 0   | 0   | +   | +   |
| Constipation           | +++   | +   | +++   | ++  |

polyuria 9 hours after injection. The day after injection there was a noticeable diminution in the edema of the eyelids in both instances; the patients looked brighter and moved a little more quickly. The second patient, after the second injection (basal metabolic rate before injection minus 13 per cent) developed a headache in about 4 to 6 hours, and after the third injection (basal metabolic rate before injection minus 6 per cent), a headache which had been present for several days, became worse 4 hours later and she became nauseated.

The height of the intoxication was reached on the second day following all injections except the second one in the first patient. In this instance, it was reached on the fourth day after injection. In both patients at the myxedematous level, all the symptoms of intoxication had begun to abate after the fifth day. Following the second injection in the first patient, the peak of the reaction was over in six days and following the last two injections into the second patient, the symptoms attributable to intoxication had practically disappeared by the fifth day.

When the patients were myxedematous, the clinical benefit of the injection of 10 mgm of thyroxin was not at its height until three weeks after injection, and at this time the weight was still falling although the basal metabolism had dropped to minus 20 per cent in case 1 and minus 28 per cent in case 2. In the first patient, whose hospital routine was not disturbed, this benefit continued unabated for three more weeks, by which time she had lost a little more weight and her basal metabolism had fallen to minus 28 per cent. In both patients the reversion to myxedema was very gradual and by no means complete. When their basal metabolic rates had returned to their pre-injection levels of minus 40 and minus 45 per cent respectively, both patients showed very few manifestations of thyroid deficiency and moreover weighed 6 and 5 kilograms less respectively than before injection, although at these same levels of metabolism they had previously been classical cases of myxedema. Table 2 illustrates this point.

## DISCUSSION

### *A Differences in excess heat production*

That the decrease in the magnitude of the reaction to the intravenous injection of 10 mgm of thyroxin at a normal level of basal

metabolism as compared with a myxedematous level of metabolism, is not due to the establishment of a tolerance to thyroid substance, is upheld by the following considerations

1 We have never encountered a case of myxedema, in which a dose of desiccated thyroid which was definitely established by administration for a long enough period of time to be sufficient for maintenance, had to be increased later because of the development of a tolerance to the substance.

2 The response of normal and obese individuals to desiccated thyroid by mouth (1) (3) (4) (5) or to thyroxin given intravenously (2) (6) is less than that of patients with myxedema

A more reasonable explanation of the decrease observed, is that at the myxedematous level, the thyroxin is supplying a marked, perhaps complete deficiency of thyroid gland secretion, whereas at a normal level, thyroxin is being given in excess. The body would be expected to deal with these different situations in a different manner, in the latter instance bringing into play some mechanism which would tend to keep conditions constant

By injecting the thyroxin intravenously, it can be made certain that it is completely absorbed by the blood stream but the amount, time and method of its absorption by other tissues and its excretion from the body, under different circumstances, are practically unknown. Kendall (7) states that within fifty hours after 200 milligrams were injected into the saphenous vein of a dog, 43 per cent of the total iodine contained in the thyroxin was excreted in the bile and 13 per cent in the urine. In this case the thyroxin was given in great excess so that these figures probably do not represent the per cent of a 10 mgm dose that is excreted unused when given to a patient with myxedema.

It is reasonable to assume that under normal conditions, when the rate of cell metabolism is more rapid than in the myxedematous condition, the excretion of thyroxin may also be more rapid. This may be at least one element in the mechanism which, at a normal level of metabolism, guards against the effects of excess dosage of thyroxin.

Incidentally, a curve denoting the effect on basal metabolism of an intravenous dose of thyroxin represents only the effect of whatever fraction of that dose is not eliminated from the body unused at one

time or another during the course of the observations. One would expect this fraction to vary under different circumstances.

Plummer and Boothby (8) state that in myxedema, 1 milligram of thyroxin injected intravenously raised the basal metabolism an average of 2.8 per cent. Their table shows that they divided their myxedematous patients into groups according to the metabolism, which ranged from below minus 35 per cent to above minus 14 per cent. The lower the metabolism, the larger the dose of thyroxin administered. Their results ranged irregularly from 1.6 to 3.5 per cent increase in metabolism per milligram of thyroxin injected. In the groups with metabolic rates "above minus 14 per cent," "minus 15 to minus 19 per cent" and "minus 35 per cent and below" (the only ones in which significantly different doses were given at the same metabolic level), the results suggest that the greater the dose the less the increase in metabolism per milligram of thyroxin injected. That the size of the dose may affect such results is indicated by Kendall's (9) observation that the injection of one enormous dose (200 mgm.) into a dog had no more clinical effect than a single small dose. While 2.8 per cent rise per milligram is the proper *average* for Plummer and Boothby's observations throughout their metabolic range, and may be for such observations in general, it should be understood that the variations allowed for by this figure are within rather wide limits. Our findings indicate that for a 10 mgm. dose, the variation may range from a rise of 4.3 per cent per milligram to a rise of only 2 per cent per milligram, depending upon the initial level of the basal metabolism within the range of minus 45 to minus 4 per cent.

Boothby, Sandiford, Sandiford and Baldes (10) have attempted to reduce the relation between the size of the dose and the excess heat production to mathematical terms. On the basis of their interesting data most of which are charted in another article (2), and which were made on one myxedematous patient, they tentatively estimate that the continued administration of an average intravenous dose of 0.25 mgm. of thyroxin daily raised the heat production approximately 525 calories above the myxedematous basal level of 1100 calories, an average dose of 1 mgm. daily raised it approximately 825 calories and 2 mgm. daily, approximately 1020 calories. As this relation between

dose and extra heat is a straight line on logarithmic paper, they suggest the following equation to represent it

$$\text{Log } H - n \log T = K$$

where  $H$  represents the excess calories and  $T$  the daily dose of thyroxin necessary to maintain  $H$

As the authors evidently realize however, judging by the fact that they offer it as only a preliminary suggestion, there are possibilities for error in this generalization. The most serious criticism of their formula is that the actual doses were not 0.25, 1 and 2 mgm daily, but averages of different doses given at longer intervals, viz., 1 to 14 days. The results of such a program of medication may differ from the results that would be obtained from actually giving 0.25, 1 and 2 mgm daily, on account of possible differences in the rate of excretion of thyroxin. Moreover, the per cent of thyroxin excreted unused when the dose is 2 mgm daily may be different from that excreted unused when the dose is smaller. Another consideration is that there may be a different relationship between excess heat and dose at levels of basal metabolism below standard normal, from that which obtains above normal.

### *B Differences in length of descending portions of the curves*

Plummer (6) makes the general statement that, in a thyroidless individual, the effect on basal metabolism of an intravenous injection of thyroxin—sufficient to bring the metabolism to normal—wears off in 5 to 7 weeks. In another article (11), he states that the effect of 14 mgm. may not have completely worn off in 8 weeks. Boothby, Sandiford, Sandiford and Slosse (2) report two curves on a myxedematous patient. The first represents the rate of decrease of the metabolism which had been raised by 3.9 grams of desiccated thyroid by mouth, and occupies a minimum of 45 days. The other represents the rate of disappearance of the effect on metabolism of an average intravenous dose of 1 mgm of thyroxin daily for about a month, and occupies a theoretical minimum of about 55 days. The curve is not plotted on actual data in the last half of its course. In another article (12) Boothby and Sandiford state that the time required for the heat production to decrease from the maximum to one-

tenth of this amount, is found by extrapolation to be usually between 30 and 70 days for thyroxin W A Plummer (13) states that the effect of an intravenous injection of thyroxin on metabolism in myxedema sometimes lasts for 10 weeks Baumann and Hunt (14) on the basis of their experiments on "the specific dynamic action of glucose" following complete thyroidectomy in rabbits, concluded that it took about 65 days for the thyroid secretion in the tissues to become exhausted Magnus Levy (15) found in his myxedematous patient that it took about 80 days for the effect of thyroid feeding on basal metabolism to wear off

As may be seen from figures 3 and 4, the level of the metabolism at the time of injection of thyroxin has a very important bearing on the time occupied by the decrease in basal metabolism, and consequently upon the total amount of excess heat produced This may explain some of the differences noted by various observers in the duration of the descending portion of the curve

*C Possible significance of the lag of clinical effects behind basal metabolism after injection of thyroxin when the patients were myxedematous*

The marked lag in disappearance and reappearance of clinical signs and symptoms of myxedema behind the rise and fall in basal metabolism illustrates the striking slowness with which the chemical changes in tissues that are characteristic of myxedema occur In terms of basal metabolic rate, the effect of the injection of 10 mgm of thyroxin in myxedema is completed in 90 days, but in terms of general well-being, the effect lasts for some time longer—probably several months If the latter effect be due to some residual thyroxin in the tissues, then the rate of destruction of thyroxin has never been even approximately determined If it is merely an expression of some mechanism which thyroxin has set into play and which is acting long after the thyroxin itself has become exhausted, then there is no guarantee that at least part or possibly all of the descending portion of the basal metabolism curve is not of the same nature Possibilities such as these make one hesitate to apply formulae to the descending parts of the curves of metabolism and

to use the results to calculate the amount of thyroxin in the body<sup>4</sup> Even if there were sufficient data, well controlled as to dosage and level of metabolism at the time of injection, one would be at a loss to know at what point all the thyroxin actually disappeared from the reaction

Boothby, Sandiford, Sandiford and Baldes (10) state that the data of the descending portions of their three curves on one myxedematous patient lie within the experimental error of an exponential curve of the following formula

$$H + H' = 1660 \times 10^{-0.024t} + 1150$$

where  $H$  = excess heat calories due to thyroxin and 1660 is an arbitrary value for zero day,  $10^{-0.024t}$  is the exponential constant,  $t$  the number of days following the zero day and  $H'$  is 1150, the heat production of the myxedematous subject before the administration of thyroxin The excess heat production when plotted on semi-logarithmic paper against time fell on a straight line and could be expressed by the above formula. Therefore, they tentatively assumed that this excess heat was dependent on some function of the concentration of thyroxin in the body They further state that "if we assume that the excess heat is directly proportional to the concentration of thyroxin in the body then the disappearance of thyroxin from the body obeys the same law as the excess heat production and the rôle of the thyroxin is identical with that of a specific catalyst and follows the law of monomolecular reactions"

Rabinowitch (16) points out that while the descending portion of the curve possibly may be explained on such a basis, the ascending portion cannot be. "Since we are dealing with a reaction which increases in magnitude for a number of days after the administration of a single dose of the drug, it is obvious that the concentration of that

<sup>4</sup> Inasmuch as the rate at which thyroxin is used and the magnitude of its effect appear to depend upon the level of the basal metabolism before injection and the size of the dose given, inasmuch as the basal metabolism does not necessarily record the concentration of thyroxin in the tissues, and inasmuch as the rate and time at which it disappears from the reaction when its administration is stopped cannot be determined at present, we find it difficult to understand how Plummer (11) calculated that there are about 14 mgm. of thyroxin in the body of a normal man His method of making this calculation is not recorded.

drug in the tissues is not the only governing factor" He suggests that we are dealing with a form of growth in which the rate of increment is at any particular instant proportional to the magnitude of that which is increasing Thus the successive rates of heat production at the end of a series of intervals would form the terms of a geometrical progression, according to the formula

$$Q_t = Q_o e^{kt} \text{ or } k = \frac{2.302}{t} \log_{10} \frac{Q_o + X_t}{Q_o}$$

where  $Q_o$  = the original quantity,  $k$  = constant,  $Q_t$  = the amount to which  $Q_o$  has grown in time  $t$  and  $X_t$  = the increment during time  $t$  "If the law is applicable, it should be possible to predict the heat production at the end of any period of observation, once the constant of increment is known" When a constant value for  $k$  was used throughout the period of increment, the calculated results agreed reasonably well with the observed results If values for  $k$  were calculated at different intervals, however, a marked inconstancy was noted He found a similar variation in the value for  $k$  in the descending portion of one of the curves of Boothby and Rowntree (17) He therefore concluded that "we are either not dealing with a reaction that follows the unimolecular law, or, as in the case of ferments, the fall in  $k$  may be attributed to destruction of the catalyst If, however, the latter explanation is accepted, it is difficult to reconcile the increase in the rate of heat production which follows with the view that the heat production is a direct function of the concentration of thyroxin in the tissues" He suggests the possibility that, during the reaction, certain compounds are formed which are catalytic and may account for the prolonged effect following the administration of thyroxin

The slope of the descending portions of our curves would appear to agree with the contention of Rabinowitch that the reaction is not a monomolecular one and we are of the opinion that it is at present hopeless to give a satisfactory theoretical and mathematical treatment of so complex a biological phenomenon

#### *D Bearing of our results on the treatment of myxedema*

We have frequently noticed that after the basal metabolism has been raised to a normal level in patients with myxedema, it is often possible,

by increasing slightly the oral dose of thyroid ( $\frac{1}{2}$  to  $1\frac{1}{2}$  grains of Armour's thyroid daily), to produce unpleasant clinical symptoms without much increase in basal metabolism. For instance, in our first patient, 3 grains of desiccated thyroid were continued daily for nearly five months after the second injection of thyroxin. As shown in figure 1, after the effects of the injection had worn off, although the basal metabolism remained approximately normal (plus 2 to plus 4 per cent, with the exception of one determination of plus 13 per cent shortly after she went home), her weight dropped steadily. She developed some palpitation and precordial pain, was nervous, fatigued easily, perspired more than usual and looked worn out. On reducing the dose of desiccated thyroid to  $1\frac{1}{2}$  grains daily, her metabolism was still within normal limits (minus 9 to minus 2 per cent), and she gained weight. Her symptoms of thyroid intoxication disappeared and there was no evidence of myxedema.

Our results with thyroxin are in harmony with this observation. The frequent lack of effect on metabolism of a slight excess of desiccated thyroid when given at a normal level of metabolism is similar to the very moderate effect produced at this level by a large excess (10 mgm) of thyroxin.

Inasmuch as a small excess of thyroid is not necessarily reflected in the level of the basal metabolism, the latter is not the only factor to be considered in gauging the proper maintenance dose for a patient with myxedema. The determination of the dose of desiccated thyroid must be in part based upon a careful clinical study of the patient.

#### SUMMARY

In two patients with myxedema, the intravenous injection of 10 mgm of thyroxin produced more marked effects at a low level of metabolism (minus 40 and minus 45 per cent) than at a normal level of metabolism (minus 4 and minus 6 per cent respectively). This is shown by the following facts:

- 1 The total excess heat produced was about seven times as great viz., 22,455 calories vs. 3,320 calories in the first case, and 21,075 vs. 2,815 calories in the second case.

- 2 The per cent rise in basal metabolism per milligram of thyroxin

injected was about twice as much, viz , 4 per cent per milligram vs 2 per cent per milligram

3 The length of time occupied by the descending portion of the metabolism curve was about four times greater, viz , 80 to 85 days vs 18 to 19 days

4 The unpleasant clinical effects, including elevation of temperature, were more marked

5 The beneficial clinical effects were marked at the myxedematous level At the normal level, the only effect was a toxic one

The appearance and disappearance of the clinical improvement lagged considerably behind the rise and fall in basal metabolism when the injection was given at the myxedematous level

The time occupied by the ascending portions of the curves varied from 4 to 6 days, and appeared to be approximately the same at the normal level as at the myxedematous level

The slope of the descending portions of our basal metabolism curves following the injections at the lowest levels of metabolism, does not support the hypothesis of Boothby et al that the reaction is a monomolecular one

#### CONCLUSIONS

The level of the basal metabolic rate at the time of the intravenous injection of a given dose of thyroxin into a myxedematous patient, has a marked influence upon the amount of excess heat produced and upon the intensity of both the unpleasant and the beneficial clinical effects

The multiplicity of unmeasured factors involved in the reaction produced by thyroxin makes it impossible at present to reduce the effect to accurate mathematical terms The most important of these factors appear to be the amount of injected thyroxin that is effective, the nature of the chemical changes produced by thyroxin, and the time and rate at which thyroxin disappears from the reaction—a factor which may not be accurately gauged by changes in the basal metabolism

The clinical condition of the patient is just as important as the basal metabolic rate in determining the proper maintenance dose of thyroid for a patient with myxedema

## BIBLIOGRAPHY

- 1 Thompson, W O , J Clin Invest., 1926, ii, 477 Studies in Blood Volume. I. The Blood Volume in Myxedema, with a Comparison of Plasma Volume Changes in Myxedema and Cardiac Edema.
- 2 Boothby, W M , Sandiford, I , Sandiford, K., and Slosse, J , Trans Assoc. Am. Phys., 1925, xl, 195 The Effect of Thyroxin on the Respiratory and Nitrogenous Metabolism of Normal and Myxedematous Subjects I. A Method of Studying the Reserve or Deposit Protein with a Preliminary Report of the Results Obtained
- 3 Magnus-Levy, A., Ztschr f klin Med , 1897, xxxiii, 269 Untersuchungen zur Schilddrüsenfrage. Gas- und Stoffwechseluntersuchungen bei Schilddrüsenfütterung, Myxödem, Morbus Basedowii und Fettleibigkeit.
- 4 Thiele, O , and Nehring, O , Ztschr f klin Med , 1896, xxx, 41 Untersuchungen des respiratorischen Gaswechsels unter dem Einflusse von Thyreoidapräparaten und bei anämischen Zuständen des Menschen.
- 5 Jaquet, A and Svenson, N , Ztschr f klin Med., 1900, xli, 375 Zur Kenntniss des Stoffwechsels fettstüchtiger Individuen
- 6 Plummer, H S , J Am. Med Assoc., 1921, lxxvii, 243 Interrelationship of Function of the Thyroid Gland and of its Active Agent, Thyroxin, in the Tissues of the Body
- 7 Kendall, E. C , Endocrinology, 1919 iii, 156 The Physiologic Action of Thyroxin.
- 8 Plummer, H. S , and Boothby, W M , Am J Physiol., 1921, iv, 295 Specific Dynamic Action of Thyroxin
- 9 Kendall, E C , Ann Clin Med , 1922-23, i, 256 The Chemistry and the Pharmacologic Action of Thyroxin.
- 10 Boothby, W M , Sandiford I , Sandiford, K., and Baldes, E J , Abstr of Commun to the VIIth Internat. Physiol Congress held at Stockholm, Aug 3-6, 1926 Skandinav Archiv 1926, xlix, 99 Metabolism Studies Showing the Effect of Desiccated Thyroid and Thyroxin on a Patient with Myxedema.
- 11 Plummer, H. S , Oxford Medicine, Vol. iii, part ii, p 839 Functions of the Normal and Abnormal Thyroid Gland.
- 12 Boothby, W M , and Sandiford, I , J Biol Chem , 1924, lxx, p xl. A quantitative Estimate of the Catalytic Power of Adrenalin and Thyroxin as Calorigenic Agents and the Relative Rate of their Destruction
- 13 Plummer W A., Ann. Clin. Med , 1923, i, 259 Clinical Features of Thyroid Disease.
- 14 Banmann, E J , and Hunt, L , J Biol. Chem. 1925, lxxiv, 709 On the Relation of Thyroid Secretion to Specific Dynamic Action.
- 15 Magnus-Levy, A., Ztschr f Klin Med. 1904 lii, 201 Ueber Myxödem
- 16 Rabinowitch, I M , J Biol Chem. 1924-25, lxxi, 245 On the Action of Thyroxin
- 17 Boothby, W M , and Rowntree, L G , J Pharm. and Exper Therap , 1923-24, xxli, 99 Drugs and Basal Metabolism



# THE ACTION OF SODIUM CHLORIDE, AMMONIUM CHLORIDE, AND SODIUM BICARBONATE ON THE TOTAL ACID BASE BALANCE OF A CASE OF CHRONIC NEPHRITIS WITH EDEMA

BY FULLER ALBRIGHT<sup>1</sup> AND WALTER BAUER<sup>2</sup>

(From the Metabolism Ward of the Massachusetts General Hospital, Boston)

(Received for publication January 14 1929)

## INTRODUCTION

The steps in the clarification of the pathological physiology associated with the edema in the nephrotic type of chronic nephritis form an interesting and as yet unfinished chapter in modern medicine. In 1902 Widal (1) pointed out that the failure to excrete water is associated with a difficulty in excreting sodium chloride and advocated a low salt diet. In 1909 Blum (2) amplified this by showing that it is only the sodium ion which the kidney has difficulty in excreting. In 1911 Meyer and Cohn (3) observed the diuretic action of calcium chloride in infants, and in 1918 Schultz (4) used this drug in treating war nephritis. Blum, Aubel, and Hausknecht (5) in 1922 thought that the liberation of edema following the administration of calcium chloride is the result of an antagonism between calcium and sodium which facilitates the excretion of sodium. A year later Haldane, Hill, and Luck (6) reported the production of an acidosis by the ingestion of calcium chloride and attributed its diuretic action to this. In the same year Gamble, Ross, and Tisdall (7) showed that this acid effect is due to a greater absorption of the chlorine ion than of the calcium ion. In the meantime, Haldane (8) in 1921 had demonstrated that the ingestion of ammonium chloride produces an acidosis. This he attributed to the transformation of the ammonia into urea, which leaves the acid radicle chlorine to unite with body base. In

<sup>1</sup> Research Fellow, Massachusetts General Hospital and Harvard Medical School.

<sup>2</sup> Medical Resident, Massachusetts General Hospital.

1923, Keith, Barrier, and Whelan (9) drew attention to the diuretic action of ammonium chloride in nephritis with edema. Finally in 1925, Gamble, Blackfan, and Hamilton (10) showed that calcium chloride and ammonium chloride bring about a diuresis by producing an acidosis. They pointed out that in order to excrete the chlorine ion there must be a corresponding increase in the titratable acidity of the urine and in the excretions of ammonia and total base. The first two of these factors do not increase sufficiently to take care of the increased chlorine excretion, and therefore base is withdrawn from the body fluids. This base they found to be sodium and potassium. With the increased excretion of base, the water held by the base in the body is liberated, and diuresis results. This excellent paper practically limits the possibilities for clarification in this direction but leaves two things to be explained. What is the cause of the original difficulty in excreting base? Granted that acid salts act by making necessary an increased excretion of base to prevent severe acidosis, what is the exact physiological means by which they accomplish this? In other words, we cannot be content with the explanation that the body has to excrete base when an acid salt is taken. We should like to know just how this is brought about.

An entirely different approach to this question starts with the work of Epstein (11) (12) who emphasized the low albumin of the serum, the low protein content of edema and ascitic fluid, and the high fat content of the blood as indicated by the high cholesterol. He believed that the hypoproteinemia is the basic disorder and directed his treatment toward the relief of this. Salvesen and Linder (13) in 1923 showed that with the lowered serum protein in nephritis there is a corresponding lowered serum calcium, and that the calcium content of various body fluids bears a definite relation to the protein content. Recently Blackfan and Hamilton (14) have made what is probably a very significant contribution when they demonstrated a low concentration of total base in the serum of children suffering with nephrosis.

In summary, we have a condition associated with massive albuminuria, a resulting low serum albumin, a low serum calcium dependent on the low serum protein, a low serum total base possibly also dependent on the low serum protein, a difficulty in excreting base in the urine, a resulting accumulation of base in the tissues, a retention of

water to keep the relation of base to water in the body fluids constant, and hence edema

The present investigation was undertaken in order to test the validity of the above concept, and to discover, if possible, additional relations

#### EXPERIMENTAL DATA

A patient with relatively mild, almost stationary, and essentially uncomplicated "nephrosis" was selected who was willing to cooperate in this rather tedious investigation. The details of the clinical history are appended. In brief, she was an adult woman with massive albuminuria, marked edema, low serum albumin, high blood cholesterol, normal renal function as shown by the excretion of phenol-sulphophthalein, normal blood non-protein nitrogen, normal urinary sediment, and normal blood pressure.

She was transferred to the Metabolism Ward where she was studied for twelve three-day metabolism periods. During this entire time (36 days) she received a carefully weighed diet<sup>3</sup> containing exactly the same articles of food every day. Likewise, the same amount of fluid was administered every day at the same hours. She was allowed out of bed for exactly the same amount of time every day. The urine and feces were collected in three day periods for analysis. Venous blood for analysis was withdrawn under oil at frequent intervals. All determinations were made on serum.

The urine was analyzed for what we have termed "titratable acidity minus  $\text{CO}_2$ ," ammonia, total base, calcium, phosphorus, sulphates, chlorine, protein, nitrogen, and non protein nitrogen, the feces for calcium, total base, and phosphorus, the serum for total base, calcium, protein, albumin, globulin,  $\text{CO}_2$  combining power, chlorine, and phosphorus. The methods employed in the collection of the excreta, the preparation of the diet, and the analyses of the serum, urine, and feces have been given in a previous paper (15). The titratable acidity minus  $\text{CO}_2$  and the ammonia were analyzed daily. The value of the former expression was obtained by adding a known amount of acid to the urine, shaking until the  $\text{CO}_2$  was entirely driven off, and then

<sup>3</sup> The details of the diet are appended.

titrating back to a pH of 7.35. The amount of alkali minus the amount of acid added equals the "titratable acidity minus  $\text{CO}_2$ ." In an alkaline urine this may become a negative expression.

The investigation consisted of a sequence of three control periods (9 days), two in which salt was added to the diet, two in which this was replaced by ammonium chloride, one control period, one in which sodium bicarbonate was administered, and finally three control periods.

## RESULTS

### *A Total base<sup>4</sup> metabolism (See table 1 and chart 1)*

In chart 1 there are given graphically the data for the total base exchange, the output of urine, and the body weight. Since body fluids contain approximately the base equivalent of 150 cc of N/10 base per 100 cc (16), the retention of 150 cc of N/10 base should be associated with the retention of 100 cc of fluid and a gain in weight of 100 grams. This of course is true, provided the patient is in nitrogen balance. (An insignificant error enters into this reasoning due to the deposit of base as calcium in the bones). Therefore, in chart 1 the scale for the weight is chosen such that 1000 grams on the weight scale is equivalent to 1000 cc of N/10 base on the base scale. Likewise, the urine is charted on a scale of the same size as the weight.

An examination of chart 1 shows that, during control periods 1, 2, and 3 on a low base intake, the patient was approximately in total base equilibrium and that the weight and urinary output were almost stationary. During periods 4 and 5, in spite of a marked increase in the total base intake, due to the ingested sodium chloride, there was no increase in the total base output except for a slight rise in the urinary base in period 5. The entire added base was retained in the body, the body weight rose and the amount of urine decreased. During periods 6 and 7, when the chlorine was added in the form of ammonium chloride instead of sodium chloride, there was a marked increase in total base excretion with a negative total base balance and a corresponding increase in output of urine and decrease in body weight. In control period 8 there was a return to equilibrium. During period 9,

<sup>4</sup> By total base is meant the sum of calcium, magnesium, sodium, and potassium expressed in cubic centimeters of N/10.

when the base was administered in the form of sodium bicarbonate in place of sodium chloride, there was a marked rise in the total base

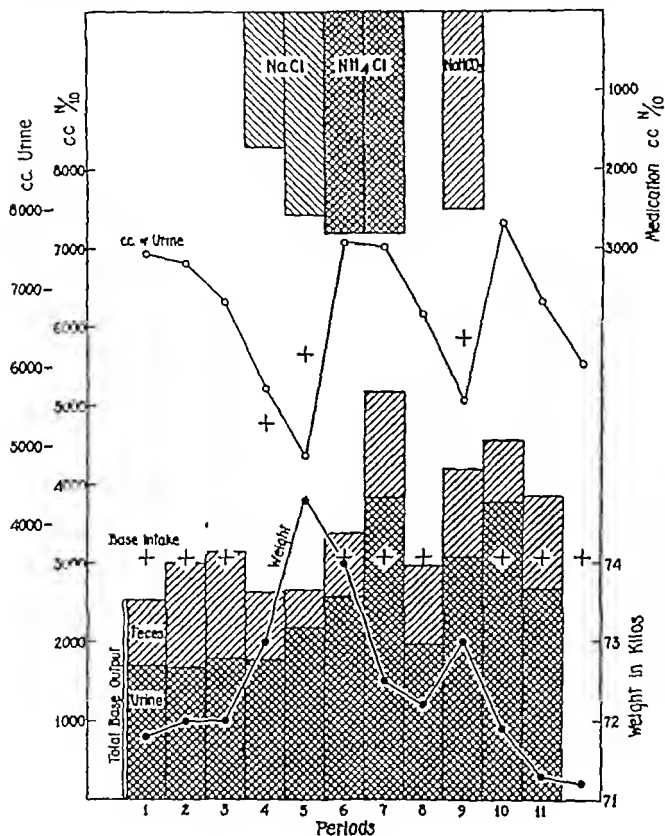


CHART 1 GRAPHIC REPRESENTATION OF DATA FROM TABLE 1

excretion, not, however, equal to the increased total base intake. Corresponding to the retained base, there was a decrease in output of

urine and an increase in weight. In control period 10, the increased base excretion continued. During period 11, equilibrium was again reached. From the last two columns of table 1 it will be noted that the change in weight from period to period corresponded extremely well with the change in weight as calculated from the total base balance.

TABLE 1

*The data for the total base balance, output of urine and body weight for the 36 days of observation*

It will be noted that the actual change in weight from period to period corresponds extremely well with the change in weight as calculated from the total base balance.

| Period | Total base |            |            |              |            | Urine | Weight | Medication                  | Gain in weight | Gain in weight calculated from base balance |
|--------|------------|------------|------------|--------------|------------|-------|--------|-----------------------------|----------------|---|
|        | Intake     | Urine      | Feces      | Total output | Balance    |       |        |                             |                |   |
|        | cc of N/10 | cc of N/10 | cc of N/10 | cc of N/10   | cc of N/10 | cc    | kgm    | cc of N/10                  | kgm            | kgm   |
| 1      | 3,087      | 1,700      | 842        | 2,542        | +545       | 7,445 | 71.8   |                             | +0.2           | +0.3  |
| 2      | 3,087      | 1,686      | 1,327      | 3,013        | +74        | 7,320 | 72.0   |                             | +0.2           | 0.0   |
| 3      | 3,087      | 1,795      | 1,358      | 3,153        | -66        | 6,825 | 72.0   |                             | 0.0            | 0.0   |
| 4      | 4,797      | 1,782      | 852        | 2,634        | +2,163     | 5,725 | 73.0   | 1,710 of NaCl               | +1.0           | +1.4  |
| 5      | 5,652      | 2,178      | 483        | 2,661        | +2,991     | 4,880 | 74.8   | 2,565 of NaCl               | +1.8           | +2.0  |
| 6      | 3,087      | 2,569      | 816        | 3,385        | -298       | 7,590 | 74.0   | 2,800 of NH <sub>4</sub> Cl | -0.8           | -0.2  |
| 7      | 3,087      | 3,890      | 1,289      | 5,179        | -2,092     | 7,530 | 72.5   | 2,800 of NH <sub>4</sub> Cl | -1.5           | -1.4  |
| 8      | 3,087      | 1,966      | 1,005      | 2,971        | +116       | 6,665 | 72.2   |                             | -0.3           | +0.1  |
| 9      | 5,587      | 3,083      | 1,110      | 4,193        | +1,394     | 5,570 | 73.0   | 2,500 of NaHCO <sub>3</sub> | +0.8           | +0.9  |
| 10     | 3,087      | 3,775      | 784        | 4,559        | -1,472     | 7,820 | 71.9   |                             | -1.1           | -1.0  |
| 11     | 3,087      | 2,675      | 1,179      | 3,854        | -767       | 6,820 | 71.3   |                             | -0.6           | -0.5  |
| 12     | 3,087      |            | 942        |              |            | 6,020 | 71.2   |                             | -0.1           |   |

From the data thus far, the following points seem significant:

1. Gains and losses of edema fluid could be approximately predicted from the total base balances on the supposition that 100 cc of tissue fluids are equivalent to 150 cc of N/10 base.

2. Addition of the neutral salt sodium chloride for a period of six days resulted in an almost quantitative retention of the added base.

3. In spite of inability to excrete base, the absorption of sodium chloride was quantitative.

4. Addition of the acid-producing salt, ammonium chloride, increased the total base excretion in the urine and this increase was

more marked during the second three days than during the first three days. This effect stopped immediately on cessation of the drug.

5. Addition to the diet of the alkaline salt, sodium bicarbonate, increased the total base excretion but not sufficiently to offset the increased base intake. This increased excretion continued and even increased during the first control period following the sodium bicarbonate period.

TABLE 2  
*Data showing the calcium and phosphorus balances*

| Period | Calcium        |                |                |                | Phosphorus     |                |                |                | Medication                      |
|--------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|---------------------------------|
|        | Intake         | Urine          | Feces          | Balance        | Intake         | Urine          | Feces          | Balance        |                                 |
|        | cc. of<br>N/10 | cc. of<br>N/10 | cc. of<br>N/10 | cc. of<br>N/10 | cc. of<br>N/10 | cc. of<br>N/10 | cc. of<br>N/10 | cc. of<br>N/10 | cc. of N/10                     |
| 1      | 154            | 17             | 208            | -71            | 1,885          | 1,275          | 496            | +114           |                                 |
| 2      | 154            | 18             | 250            | -114           | 1,885          | 1,022          | 774            | +89            |                                 |
| 3      | 154            | 36             | 228            | -110           | 1,885          | 990            | 703            | +192           |                                 |
| 4      | 154            | 19             | 153            | -18            | 1,885          | 2,270          | 502            | -887           | 1,710 of NaCl                   |
| 5      | 154            | 28             | 129            | -3             | 1,885          | 1,179          | 624            | +82            | 2,565 of NaCl                   |
| 6      | 154            | 77             | 146            | -69            | 1,885          | 1,445          | 504            | -64            | 2,800 of $\text{NH}_4\text{Cl}$ |
| 7      | 154            | 110            | 195            | -151           | 1,885          | 5,120          | 611            | -3846          | 2,800 of $\text{NH}_4\text{Cl}$ |
| 8      | 154            | 81             | 147            | -74            | 1,885          | 712            | 425            | +748           |                                 |
| 9      | 154            | 27             | 171            | -44            | 1,885          | 922            | 505            | +458           | 2,500 of $\text{NaHCO}_3$       |
| 10     | 154            | 45             | 113            | -4             | 1,885          | 900            | 433            | +552           |                                 |
| 11     | 154            | 32             | 202            | -80            | 1,885          | 1,119          | 596            | +170           |                                 |
| 12     | 154            | 10             | 157            | -13            | 1,885          |                | 578            |                |                                 |

### *B Calcium and phosphorus metabolism*

It will next be of interest to note the calcium metabolism and to see whether this component of the total base metabolism behaves in any way similar to the total base as a whole. The basic diet was low in calcium and similar to that used in a study of the calcium excretion of normal individuals (17). In that study of thirteen normal men with an average calcium intake of 165 cc. of N/10 calcium per three-day period, there was an average output in the urine of 85 cc. of N/10 calcium, in the feces of 300 cc. of N/10 calcium, making an average negative calcium balance per three day period of 220 cc. of N/10 calcium.

In control periods 1, 2, and 3 (table 2) it will be noted that the calcium excretion in the urine was strikingly small and that the negative calcium balance on a low calcium diet was likewise small <sup>5</sup> The low calcium excretion in nephrosis has been noted by Scriver (18) It will be further noted that ammonium chloride increased the urinary excretion of calcium (periods 6 and 7) whereas Scriver found that parathormone was ineffective in accomplishing this in nephrosis (18)

The ratio of the urinary to the fecal phosphorus excretion suggests that there was no difficulty in excreting phosphorus through the kidneys The increased phosphorus excretion in the urine, as a result of ammonium chloride, was far out of proportion to the increased calcium excretion, if one were assuming that these were derived from tertiary calcium phosphate from the bones There was no corresponding breakdown of nitrogen to account for the phosphorus deficit (v infra) One must conclude that this phosphorus came from phosphorus held in tissue fluids The increased phosphorus excretion resulting from the ingestion of hydrochloric acid has been discussed by Fitz, Alsberg, and Henderson (20)

The following points from the calcium and phosphorus data seem significant

6 The urinary calcium excretion was very much lower than that of normal individuals on a similar régime and there was no corresponding increased fecal calcium excretion

7 Ammonium chloride increased the urinary calcium and phosphorus excretions without affecting that in the feces The increase in the urinary phosphorus was out of proportion to the increase in the urinary calcium

### C *Nitrogen metabolism*

Before one can calculate gains in weight as gains in edema fluid (v supra), it is necessary to know that the subject was in nitrogen equilibrium Therefore, table 3 and chart 2 have been constructed It will be noted that on a nitrogen intake of 25.9 grams per period (54 grams of protein a day) the patient was in nitrogen balance

<sup>5</sup> The low urinary excretion in chronic nephritis with edema has been confirmed in two other unpublished cases studied at the Johns Hopkins Hospital in conjunction with Dr. Read Ellsworth

This was true in spite of rather large losses of protein in the urine. The protein losses in the urine in this case were smaller than one often finds in similar cases. The nitrogen from the ammonium chloride in periods 6 and 7 appeared almost quantitatively as ammonia in the urine. From this it would appear that the acidosis produced by the conversion of the ammonia into urea, when ammonium chloride is absorbed, may be later compensated for by the reconversion of urea into ammonia when the chlorine is excreted.

TABLE 3  
*Data showing nitrogen metabolism*

| Period | Intake | Non-protein nitrogen in urine | Protein nitrogen in urine | NH <sub>3</sub> nitrogen in urine | Feces† | Balance | Medication                  |
|--------|--------|-------------------------------|---------------------------|-----------------------------------|--------|---------|-----------------------------|
|        | grams  | grams                         | grams                     | grams                             | grams  | grams   | cc. of N/10                 |
| 1      | 25.9   | 18.65                         | 4.86                      | 0.39                              | 2.59   | -0.20   |                             |
| 2      | 25.9   | 20.61                         | 6.65                      | 0.82                              | 2.59   | -3.95   |                             |
| 3      | 25.9   | 18.90                         | 4.80                      | 0.76                              | 2.59   | -0.39   |                             |
| 4      | 25.9   | 17.83                         | 4.50                      | 0.93                              | 2.59   | +0.98   | 1,710 of NaCl               |
| 5      | 25.9   | 16.64                         | 3.20                      | 0.95                              | 2.59   | +3.47   | 2,565 of NaCl               |
| 6      | 29.8   | 21.47                         | 4.59                      | 2.09                              | 2.98   | +0.74   | 2,800 of NH <sub>4</sub> Cl |
| 7      | 29.8   | 21.82                         | 4.22                      | 5.30                              | 2.98   | +0.78   | 2,800 of NH <sub>4</sub> Cl |
| 8      | 25.9   | 17.54                         | 5.35                      | 3.91                              | 2.59   | +0.42   |                             |
| 9      | 25.9   | 16.28                         | 3.02                      | 1.18                              | 2.59   | +4.01   | 2,500 of NaHCO <sub>3</sub> |
| 10     | 25.9   | 20.80                         | 5.20                      | 0.88                              | 2.59   | -2.69   |                             |
| 11     | 25.9   | 20.83                         | 4.91                      | 1.25                              | 2.59   | -2.43   |                             |
| 12     | 25.9   |                               |                           | 1.58                              | 2.59   | -1.29   |                             |

\* This is inclusive of ammonia nitrogen which is represented in a separate column.  
† Fecal nitrogen taken as 10 per cent of nitrogen intake.

From this study of the nitrogen metabolism, the following points seem important:

8 In spite of protein loss in the urine, the patient remained in nitrogen equilibrium on 54 grams of protein a day.

9 When ammonium chloride was ingested, the ammonia excretion in the urine rose proportionately to the increased intake.

#### *D Total acid-base balance of urine*

Table 4 and chart 3 have been constructed in order to study the acid-base balance of the urine during the varying conditions of this inves-

tigation In this we have followed the line of approach used by Gamble (19) with a slight modification This author, by determining

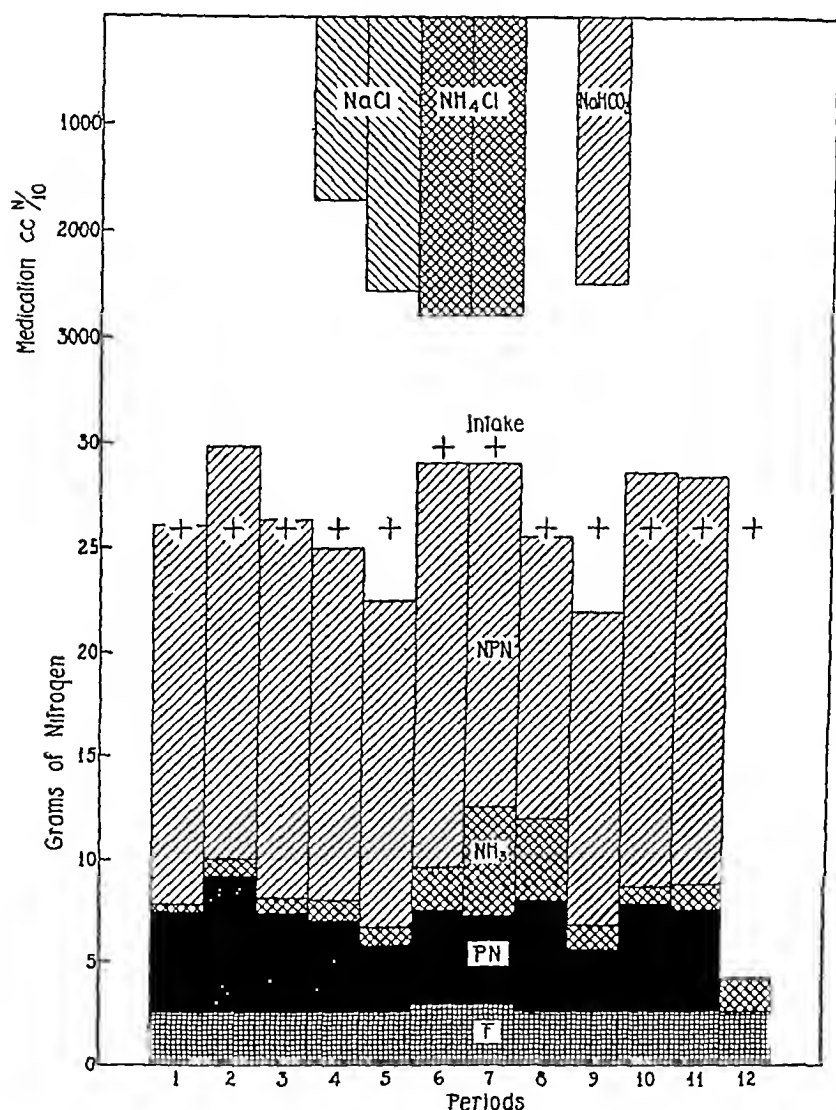


CHART 2 GRAPHIC REPRESENTATION OF DATA SHOWN IN TABLE 3

the titratable acidity of the urine ( = base necessary to bring pH of urine to 7.35), was able to construct tables and charts in which the

cations of the urine in one column were balanced by the anions in another. Thus, the sum of total base, ammonia, and titratable acidity, all expressed in cubic centimeters of N/10 equals the sum of the chlorides, sulphates, phosphates, organic acid and carbonic acid also expressed in cubic centimeters of N/10 except that a factor has to be

TABLE 4

Data for acid-base balance of the urine during the varying conditions of this investigation

| Period | Titratable acidity minus CO <sub>2</sub> | Cations in urine |               |                | Base bound by anions of urine |                 |               |                    |                                     |               | Medication                     | Chlorine intake |
|--------|--|------------------|---------------|----------------|-------------------------------|-----------------|---------------|--------------------|-------------------------------------|---------------|--------------------------------|-----------------|
|        |  | NH <sub>3</sub>  | Total base    | Sum of cations | Cl                            | SO <sub>4</sub> | P at pH 7.35  | Protein at pH 7.35 | Base bound by deter-<br>mined anion | Organic acid  |                                |                 |
|        |  |                  |               |                |                               |                 |               |                    |                                     |               |                                |                 |
|        | cc of<br>N/10                            | cc of<br>N/10    | cc of<br>N/10 | cc of<br>N/10  | cc of<br>N/10                 | cc of<br>N/10   | cc of<br>N/10 | cc of<br>N/10      | cc of<br>N/10                       | cc of<br>N/10 | cc of N/10                     | cc of<br>V/10   |
| 1      | 41                                       | 279              | 1 700         | 2 020          | 104                           | 404             | 773           | 53                 | 1 334                               | 686           |                                | 570             |
| 2      | -21                                      | 589              | 1 686         | 2 254          | 106                           | 470             | 620           | 72                 | 1 268                               | 986           |                                | 570             |
| 3      | -47                                      | 543              | 1,795         | 2 291          | 188                           | 463             | 595           | 52                 | 1 298                               | 993           |                                | 570             |
| 4      | 163                                      | 667              | 1 782         | 2 612          | 313                           | 410             | 1,370         | 49                 | 2,142                               | 470           | 1 710 of<br>NaCl               | 2 370           |
| 5      | 400                                      | 683              | 2 178         | 3 261          | 1,008                         | 358             | 709           | 35                 | 2,110                               | 1,151         | 2 565 of<br>NaCl               | 3,260           |
| 6      | 652                                      | 1,492            | 2,569         | 4 713          | 3,280                         | 451             | 864           | 50                 | 4 645                               | 68            | 2 800 of<br>NH <sub>4</sub> Cl | 3 370           |
| 7      | 684                                      | 3 785            | 3,890         | 8,359          | 4,813                         | 371             | 3,070         | 46                 | 8 300                               | 59            | 2,800 of<br>NH <sub>4</sub> Cl | 3 370           |
| 8      | 468                                      | 2,787            | 1,966         | 5 211          | 2 058                         | 358             | 428           | 58                 | 2,902                               | 2,309         |                                | 570             |
| 9      | -1 008                                   | 843              | 3,083         | 2,918          | 546                           | 318             | 554           | 33                 | 1 451                               | 1,467         | 2 500 of<br>NaHCO <sub>3</sub> | 570             |
| 10     | -1 017                                   | 630              | 3,775         | 3 388          | 716                           | 477             | 540           | 56                 | 1 789                               | 1,599         |                                | 570             |
| 11     | -57                                      | 897              | 2 675         | 3,515          | 848                           | 444             | 720           | 53                 | 2,065                               | 1,450         |                                | 570             |
| 12     | 202                                      | 1 134            |               |                |                               | 470             |               |                    |                                     |               |                                | 570             |

introduced for the phosphates, which at the pH of 7.35 bind only 1.8 equivalents of base rather than 3.

We have modified this scheme in that both our cation and our anion columns are shorter by the carbonic acid value, i.e., on the cation side our third value is the "titratable acidity minus  $\text{CO}_2$ " instead of the titratable acidity, and on the anion side we have omitted the

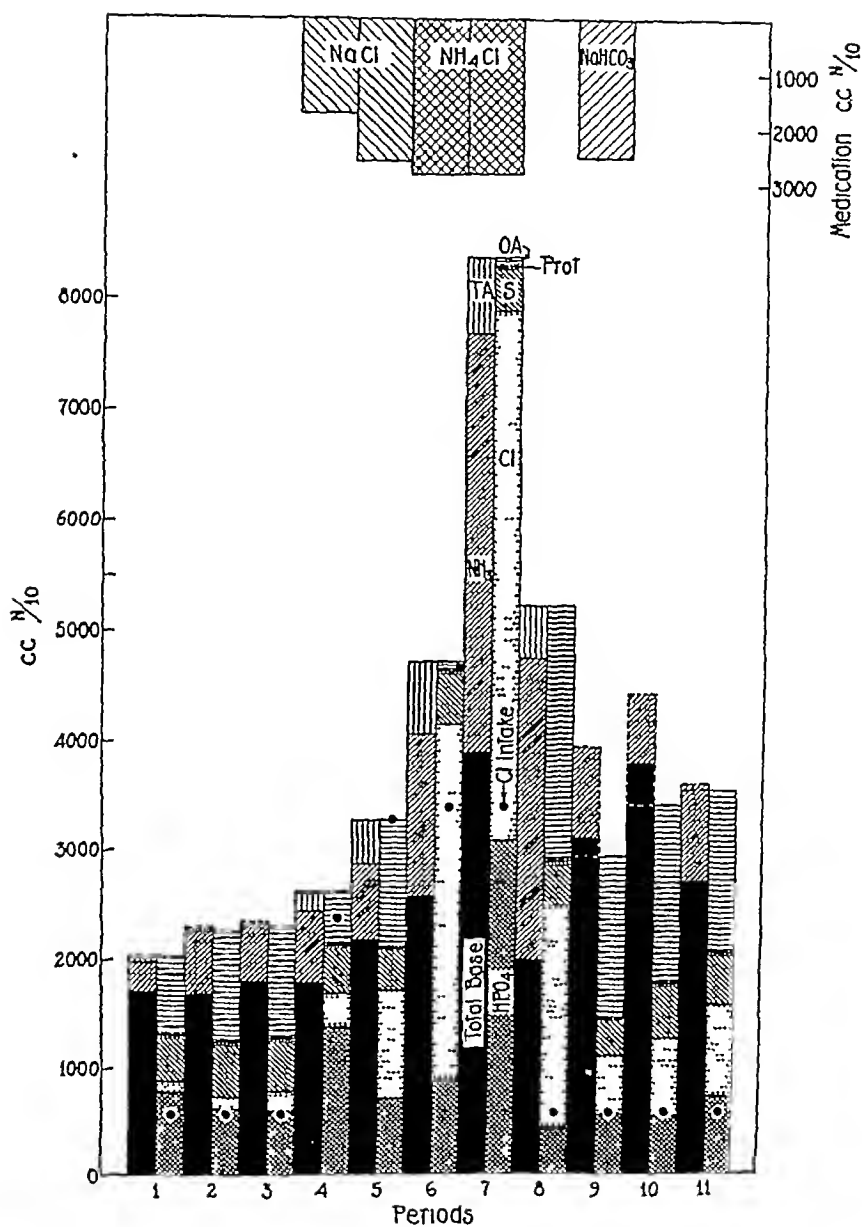


CHART 3 GRAPHIC REPRESENTATION OF DATA SHOWN IN TABLE 4

carbonic acid We have not estimated the organic acids but have assumed this to be represented by the difference between the cation column and the sum of the other anions Such values for the organic acids contain the errors of all the determinations In addition, because of the large amount of protein in the urine, we have attempted to ascertain the base-binding value of this at pH 7.35 by using the formula of Van Slyke, Wu and McLean (21) for base bound by serum protein,

$$BP = 0.63 P (\text{pH} - 4.80)$$

in which  $BP$  and  $P$ , represent respectively cubic centimeters of N/10 base per 100 cc. of serum combined with protein, and per cent of protein in the serum We appreciate that any value for base bound by urine protein thus obtained is extremely inaccurate, especially as the protein is mostly albumin rather than a mixture of albumin and globulin

It is of interest to compare the chlorine excretion in the urine with the chlorine intake (see last column in table 4) During the control periods 1, 2 and 3 the urinary excretion of chlorine was very low, about  $\frac{1}{4}$  of the intake During the sodium chloride periods the urinary excretion went up, but very much less than the intake This does not show an inability to excrete chlorine but merely represents the retention of chlorine necessary for offsetting the retained base In the ammonium chloride periods, the urinary excretion of chlorine exceeded the intake, which was to be expected The excess represents the chlorine held by the base in the edema fluid The increased chlorine excretion continued into control period 8 During the sodium bicarbonate period the urinary chlorine was again reduced, probably because chlorine was again withheld to offset the withheld base. During control periods 10 and 11 the excretion again rose above the intake concomitant with the loss of edema fluid

In chart 3, during control periods 1, 2, and 3 it will be noted that the "titratable acidity minus  $\text{CO}_2$ " was small or negative During the sodium chloride period the anion columns were increased due to a rise in the chlorine and phosphorus excretion This was compensated for in the cation column by a rise in the "titratable acidity minus  $\text{CO}_2$ " and a rise in the ammonia During the ammonium chloride periods the anion columns were very much increased due to

the marked increase in the chlorine and phosphorus excretions. This was compensated for on the cation side by a marked increase in all components. In control period 8 the findings still showed the effect of period 7, but it will be noted that the total base excretion was the first of the cation components to return to its previous level. This rather suggests that total base excretion in response to an acid is a last line of defense and only takes place after the ammonia mechanism and the titratable acidity have been taxed to their limits. During the sodium bicarbonate period, the "titratable acidity minus  $\text{CO}_2$ " became a negative quantity due to the large amount of  $\text{CO}_2$ . In this period and the following period it would appear that anions were in demand to neutralize cations which were being excreted in excess, whereas in the ammonium chloride periods, the exact opposite was the case. In other words, the increased excretion of base as a result of ammonium chloride ingestion may be thought of as an effort on the body's part to ward off fatal acidosis, whereas the increased excretion of base as a result of sodium bicarbonate is an indication that the kidneys in this case can excrete base under certain conditions.

The figures for the organic acid excretions are of interest, although it must be repeated that they contain all the errors of this investigation. They showed little of interest during the first five periods. During the ammonium chloride period there was a marked decrease in organic salt excretion, possibly because base was at a premium and it was more important to excrete the mineral acid chlorine. The increased organic acid excretion in the following control period may represent this stored organic acid. During the sodium bicarbonate period and the period following, the organic acid excretions were high, possibly because anions were in demand for excretion with the base.

From table 4 and chart 3 the following observations seem significant.

10. Provided that it was not being withheld to offset the retained fixed base, chlorine was readily excreted.

11. When additional sodium chloride was ingested, the sodium was entirely retained, part of the chlorine was withheld to offset the sodium, the rest of the chlorine was excreted in the urine with a resulting rise in the titratable acidity and the ammonium excretion.

12 Addition of ammonium chloride caused a marked rise in the chlorine and phosphorus excretion and this was offset by a rise in all the cation components of the urine

13 The increased excretion of fixed base after ingestion of acid tended to occur only after other mechanisms for the neutralization of acids had been thoroughly taxed

14 The increased excretion of fixed base after alkali ingestion apparently depended on a primary increased ability to excrete base

TABLE 5

*Data for serum electrolytes obtained at various times during this investigation*

| Period | Day of period | Calcium |             | Base bound by |                 | Chlorine    | Protein           | Base bound by protein | Base bound by total determined acid | Total base  | Organic acid | Total base excretion in urine in corresponding period |
|--------|---------------|---------|-------------|---------------|-----------------|-------------|-------------------|-----------------------|-------------------------------------|-------------|--------------|---|
|        |               |         |             | Phosphate     | CO <sub>2</sub> |             |                   |                       |                                     |             |              |   |
|        |               | mgm.    | cc. of N/10 | cc. of N/10   | cc. of N/10     | cc. of N/10 | grams per 100 cc. | cc. of N/10           | cc. of N/10                         | cc. of N/10 | cc. of N/10  | cc. of N/10   |
| 2      | 2             |         |             |               |                 |             | 4.28              | 7.43                  |                                     | 144.5       |              | 1,686   |
| 3      | 3             | 8.65    | 4.32        | 2.48          |                 |             |                   |                       |                                     | 147.0       |              | 1,795   |
| 5      | 3             | 8.15    | 4.07        | 2.28          | 27.3            | 107.5       | 3.58              | 6.20                  | 143.3                               | 157.5       | 14.2         | 2,178   |
| 7      | 2             | 8.80    | 4.40        | 2.36          | 24.9            | 112.0       | 4.55              | 7.90                  | 147.2                               | 158.0       | 10.8         | 3,890   |
| 8      | 2             | 8.50    | 4.25        | 2.32          | 28.0            | 106.0       | 4.20              | 7.30                  | 143.6                               | 149.0       | 5.4          | 1,966   |
| 9      | 3             | 8.65    | 4.32        | 2.50          | 31.4            | 103.4       | 4.30              | 7.46                  | 144.8                               | 158.0       | 13.2         | 3,083   |
| 11     | 3             | 8.30    | 4.15        | 2.02          | 26.0            | 105.8       | 3.80              | 6.60                  | 140.4                               | 148.0       | 7.6          | 2,675   |

15 Organic acids apparently were retained in the body when base was at a premium and were excreted in excess when acid was at a premium

### *E Serum electrolytes*

The serum findings are shown in table 5 and chart 4 (q v). All values are on serum. In determining the base bound by protein we used the formula of Van Slyke, Wu, and McLean (v supra) and assumed a pH of 7.35. The objections to this formula have been discussed by Peters, Bulger, Eisenman and Lee (22), but it offers an approximation which is probably satisfactory for comparative pur-

poses Base as  $\text{BHCO}_3$  was calculated by the equation of Peters, Bulger, Eisenman and Lee (22)

$$B = \frac{\text{CO}_2 - 2.85}{2.24}$$

where 2.85 volumes per cent is equal to the amount of  $\text{CO}_2$  dissolved in serum at 40 mm of  $\text{CO}_2$  tension and  $38^\circ\text{C}$ . The phosphorus in milligrams per 100 cc was reduced to cc of N/10 per 100 cc by the

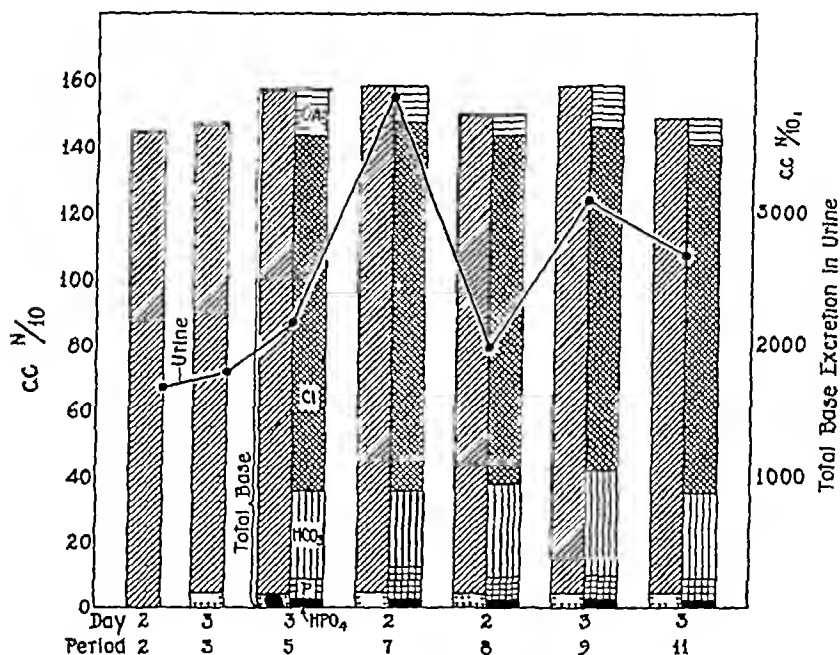


CHART 4 GRAPHIC REPRESENTATION OF DATA SHOWN IN TABLE 5

factor  $1\frac{8}{3}$ , accepting L. J. Henderson's (23) estimate of the proportion of primary and secondary phosphate in the blood. The difference between the sum of the base bound by the determined acid and the total base we have termed "organic" acid, but sulphates are included also as a component of this value.

The serum calcium was definitely low by about 1.5 mgm without any corresponding rise in serum phosphorus. This is undoubtedly due to the low serum protein as pointed out by Salvesen (13). The serum total base was likewise low during control periods 2, 3, 8, and

11 This supports the work of Blackfan and Hamilton (14). These authors give the normal range of serum total base as 154 to 161 cc. of N/10 base. It is of interest that this lowered base value rose to normal during the latter part of the sodium chloride ingestion, during the ammonium chloride period, and during the sodium bicarbonate period. When the total base value of the serum is compared with the total base excretion, it will be observed that at least in these few instances the serum total base rose as the excretion increased. During the ammonium chloride periods the  $\text{CO}_2$  fell and the chlorides rose as was to be expected. During the sodium bicarbonate period the opposite was true.

TABLE 6  
*Data for spinal fluid electrolytes*

|                        | Calcium     | Base bound by phosphorus | Base bound by $\text{CO}_2$ | Chlorine    | Base bound by protein | Base bound by total determined acid | Total base  | Organic acid |
|------------------------|-------------|--------------------------|-----------------------------|-------------|-----------------------|-------------------------------------|-------------|--------------|
|                        | cc. of N/10 | cc. of N/10              | cc. of N/10                 | cc. of N/10 | cc. of N/10           | cc. of N/10                         | cc. of N/10 | cc. of N/10  |
| Normal serum water*    | 5.10        | 2.03                     | 25.4                        | 105.0       | 12.3                  | 144.7                               | 159         | 14.3         |
| Serum water of patient | 4.45        | 2.42                     | 29.2                        | 111.0       | 7.6                   | 150.2                               | 156         | 5.8          |
| Spinal fluid           | 2.45        | 1.00                     | 23.2                        | 127.5       | 0.02                  | 121.7                               | 161         | 9.3          |

\* Values calculated from normal values given by Blackfan and Hamilton (14)

The following points here seem important:

16 The blood serum before medication differed from the normal in that the total protein was low, the total base was low, and the calcium was low.

17 Ammonium chloride, sodium bicarbonate and sodium chloride ingestion raised the serum total base.

18 The total base excretion increased in the urine when the serum total base was raised.

### *F Spinal fluid electrolytes*

A lumbar puncture was done during period 8, and it is of interest to compare the values for electrolytes found in the spinal fluid with those in the blood serum withdrawn at the same time. It is first

necessary to reduce the serum values to their values per 100 cc of serum water. The formula of Van Slyke, Wu, and McLean (21) has been used, according to which the grams of water in 1 liter of serum equal  $990 - 0.8P$ , where  $P$  is the grams of protein per liter of serum.

The spinal fluid findings are perfectly consistent with normal values. The most important difference between spinal fluid and serum is the small amount of protein in the spinal fluid. This is compensated for by increased chlorides on the anion side. The calcium in spinal fluid is likewise low due to the low protein. As the serum protein is lowered in chronic nephritis with edema, it would appear that the serum findings approach the spinal fluid findings as a limit. The spinal fluid phosphorus, as has been repeatedly shown in this laboratory<sup>6</sup>, is lower than serum phosphorus, apparently in order to keep the same ratio of Ca to P found in the blood and in the bones.

The following observations then seem important:

19 The spinal fluid electrolytes were normal.

20 The serum findings differed from normal serum findings very much as spinal fluid findings differ from serum findings only to a lesser degree.

21 The ratio of calcium to phosphorus in the spinal fluid was approximately the same as that in the blood, a relationship which we have observed in normal subjects.

It will be unnecessary to discuss all the findings here as this would lead to considerable repetition. Three points however seem worthy of emphasis.

The pathological physiology of the fixed base metabolism in chronic nephritis with edema has many points in common with that of the calcium metabolism. Thus the urinary excretion of both is low without there being any compensatory increased fecal excretion, both are low in the blood, possibly accounting for the low urinary excretion, and ammonium chloride ingestion increases the urinary excretion of both and probably raises the serum values of each.

It seems fairly well established by the work of Blackfan and Hamilton (14) that the serum total base is lowered in chronic edema. This is corroborated in the present study. It is suggested from this study

<sup>6</sup> Unpublished data.

that agents which tend to raise the lowered serum base value toward normal increase the total base excretion in the urine. In a recent contribution by Linder (24) it is of interest that one of the two cases of hydremic nephritis (case VII) had an initial total base in the serum of 135 cc. of N/10 per 100 cc., which rose to 145 cc. of N/10 under hydrochloric acid therapy. All this of course suggests that the low output of base is due to the serum base being below the threshold of kidney excretion.

The most surprising thing to us in this investigation was the response to sodium bicarbonate ingestion. It is true that the patient did become more edematous but much less so than we had expected. It would seem that the ingestion of large amounts of sodium bicarbonate likewise raises the serum base to the normal threshold of kidney excretion with a resulting increased base excretion. Our findings support the recent work of Osman (25) (26) who found that on the administration of very large doses of alkali to such patients, there was at first an increase of edema until a critical point was reached when there occurred an extraordinary diuresis.

#### SUMMARY

The total acid base balance of the urine, the fixed base, calcium and phosphorus balances of the urine and feces, frequent determinations of the serum electrolytes, and one simultaneous determination of the spinal fluid and blood serum electrolytes, have been made on a mild case of chronic nephritis with edema during twelve three day periods on a constant intake in order to determine the effect of sodium chloride, ammonium chloride, and sodium bicarbonate ingestion.

#### APPENDIX

The patient was a 35 year old married German woman who was admitted to the Massachusetts General Hospital on February 24, 1927 complaining of generalized edema.

The past history was unimportant except for measles and chicken pox in childhood, an attack of tonsillitis at the age of 15, and since then occasional sore throats.

The present illness started fifteen years ago with slight swelling of the ankles at the end of the day. This swelling gradually became more extensive. Six months before admission she noted swelling of the face.

Physical examination showed massive edema of the legs and slight edema over

the sacrum The blood pressure was 130/80 The heart was within normal limits

The urine examination showed a large amount of albumin and a normal sediment. The specific gravity varied between 1 005 and 1 015 The phenolsulphophthalein test showed a normal excretion of the dye The red blood cells were 4,800,000 and the white cells 8,600 per cubic millimeter The blood non-protein nitrogen was 25 mgm per 100 cc The serum protein was 4 28 grams per 100 cc with a serum albumin of 2 3 grams per 100 cc The blood cholesterol was 222 mgm per 100 cc The basal metabolism was minus 3 per cent

The impression was that this patient was suffering from a rather mild and quite stationary form of chronic nephritis of the nephrotic type

A letter from the patient dated June 19, 1928 states that her condition has remained stationary during the intervening year

#### *Diet*

| Food substance   | Weight       | Protein | Fat   | Carbo-<br>hydrate |
|------------------|--------------|---------|-------|-------------------|
|                  | <i>grams</i> |         |       |                   |
| Bread, salt free | 100          | 8 0     | 2 1   | 53 2              |
| Butter fat       | 40           |         | 40 0  |                   |
| Sugar            | 30           |         |       | 30 0              |
| Apple (raw)      | 200          | 0 6     | 1 0   | 27 0              |
| Orange juice     | 80           |         |       | 8 64              |
| Banana           | 100          | 1 1     | 0 6   | 21 0              |
| Steak            | 150          | 31 95   | 12 0  |                   |
| Potato           | 100          | 1 9     | 0 1   | 18 1              |
| Corn             | 100          | 2 8     | 1 2   | 19 0              |
| Tomatoes (raw)   | 100          | 1 2     | 0 2   | 4 0               |
| Chicken (white)  | 30           | 6 45    | 0 75  |                   |
|                  | <i>cc</i>    |         |       |                   |
| Coffee           | 200          |         |       |                   |
| Tea              | 400          |         |       |                   |
| Total            |              | 54 00   | 57 95 | 180 94            |

Total calories, 1461

#### BIBLIOGRAPHY

- 1 Widal, F, and Javal, A, Bull et Mém Soc Méd d Hôp de Paris, 1903, xx, 733 La cure de déchloruration
- 2 Blum, L, Verhandl d Cong f inn Med, Wiesb, 1909, xxvi, 122 Über die Rolle von-Salzen bei der Entstehung von Oedemen
- 3 Meyer, L F, and Cohn S, Ztschr f Kinderh, 1911, Orig, u, 360 Klinische Beobachtungen und Stoffwechselversuche über die Wirkung verschiedener Salze beim Saugling

- 4 Schultz, E, *Ztschr f Klin Med*, 1918, lxxxvi, 111 Klinische Beobachtungen über Nierenentzündung bei Kriegsteilnehmern
- 5 Blum, L, Aubel, E, and Hausknecht, R, *Bull et Mém Soc Méd d Hôp de Paris*, 1922, xlv, 206 L'action diurétique des sels de calcium dans la néphrite avec oedèmes
- 6 Haldane, J B S Hill, R., and Luck, J M, *J Physiol*, 1923, lvii, 301 Calcium Chloride Acidosis.
- 7 Gamble, J L Ross G S, and Tisdall F F, *Am J Dis Child*, 1923, xxv, 455 Studies of Tetany I The Effect of Calcium Chloride Ingestion on the Acid Base Metabolism of Infants
- 8 Haldane J B S, *J Physiol*, 1925 iv, 265 Experiments on the Regulation of the Blood's Alkalinity
- 9 Keith N M, Barrier, C W, and Whelan, M, *J Am Med Assoc.*, 1924, lxxxii, 666 Treatment of Nephritis and Edema with Calcium
- 10 Gamble, J L, Blackfan, K. D, and Hamilton B, *J Clin Invest.*, 1925, 1, 359 A Study of the Diuretic Action of Acid Producing Salts
- 11 Epstein, A A, *Am J Med Sci*, 1917, cliv, 638 Concerning the Causation of Edema in Chronic Parenchymatous Nephritis Method for Its Alleviation.
- 12 Epstein A A. *Med Clin N Amer*, 1920, iv, 145 Clinical Types of Chronic Parenchymatous Nephritis—Their Treatment and Results
- 13 Salvesen H. A. and Linder, G C, *J Biol. Chem.*, 1923, lviii, 617 Observations on the Inorganic Bases and Phosphates in Relation to the Protein of Blood and Other Body Fluids in Bright's Disease and in Heart Failure.
- 14 Blackfan, K D, and Hamilton B, *Bull Johns Hopkins Hosp*, 1927, xli, 322 Study of the Inorganic Constituents of the Serum in Children with Acute Nephritis
- 15 Bauer, W and Aub, J C, *J Am Diet Assoc.*, 1927, iii, 106 Studies of Inorganic Salt Metabolism I The Ward Routine and Methods
- 16 Gamble J L, Ross G S, and Tisdall F F, *J Biol Chem*, 1923, lvii, 633 Metabolism of Fixed Base during Fasting
- 17 Bauer W, Albright F and Aub J C, *J Clin Invest.*, 1929, vii, 75 Studies of Calcium and Phosphorus Metabolism. II The Calcium Excretion of Normal Individuals on a Low Calcium Diet Including Data on a Case of Pregnancy
- 18 Sriver, W deM, *J Clin Invest.*, 1928, vi, 115 Observations on the Excretion of Calcium in Two Cases of Nephrosis Treated with Parathyroid Extract.
- 19 Gamble, J L, *J Biol Chem.*, 1922, li, 295 Carbonic Acid and Bicarbonate in Urine.
- 20 Fitz R Alsberg C L, and Henderson, L J, *Am. J Physiol*, 1907, xviii, 113 Concerning the Excretion of Phosphoric Acid During Experimental Acidosis in Rabbits

- 21 Van Slyke, D D , Wu, H , and McLean, F C , J Biol Chem , 1923, lvi, 765  
Studies of Gas and Electrolyte Equilibria in the Blood V Factors Controlling the Electrolyte and Water Distribution in the Blood
- 22 Peters, J P , Bulger, H A , Eisenmen, A J , and Lee, C , J Biol Chem , 1926, lxxvii, 141 Total Acid-Base Equilibrium of Plasma in Health and Disease I The Concentration of Acids and Bases in Normal Plasma
- 23 Henderson, L J , Am J Physiol , 1908, xxi, 427 The Theory of Neutrality Regulation in the Animal Organism
- 24 Linder, G C , Quart J Med , 1927, xx, 285 The Effect of Mineral Acid on Acid-Base Regulation in Health and in Nephritis
- 25 Osman, A A , Guy's Hosp Rep , 1926, lxxvi, 412 Preliminary Observations on the Treatment of Nephritis with Oedema by Means of Large Doses of Alkalies
- 26 Osman, A A , Guy's Hosp Rep , 1927, lxxvii, 386 Studies in Bright's Disease The Use of Alkalies in Treatment of Bright's Disease and Their Prophylactic Value in This and Other Conditions Associated with Impairment of Renal Function

PROCEEDINGS OF THE TWENTY-FIRST ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N J, MAY 6, 1929

*Blood Vessels of Non-Inflammatory Origin in Human Heart Valves* By ALBERT W BROMER, and LOUISE J ZSCHIESCHE, (by invitation) and JOSEPH T WEARN, Boston, Mass

By a method of procedure not used heretofore, blood vessels have been demonstrated in one or more valves in 74 of a series of 100 human hearts. No heart with the clinical diagnosis of endocarditis or rheumatic fever has been included. A small percentage of the individuals were sufferers of arterio-sclerotic heart disease.

After as much air as possible has been drawn from the coronary system, Weber's or Higgins' India ink, diluted with an equal amount of distilled water, is injected into the coronary arteries under a pressure of 220 mm. of mercury. During the injection procedure, the heart is gently massaged in a tank of physiological saline solution at a temperature of 38°C. Inability to maintain an air tight system has probably been a source of error not infrequently.

The valve leaflets of all hearts injected have been rendered transparent by the Spalteholz method for microscopic examination. The mitral valve has presented vascularization in 58 per cent of the hearts injected, the tricuspid valve in 43 per cent, the pulmonary valve in 25 per cent, and the aortic valve in 5 per cent. Leaflets of two or more valves have been successfully injected in 39 per cent of the total number.

The presence of vessels in the valves seemingly bears no relationship to age, the number of vascularized valves of the higher decades being approximately the same as for each of the earliest decades.

The vascularization of the aortic leaflet of the mitral valve is characterized by two or more vessels of goodly calibre descending in a sweeping looping manner from the auriculo-ventricular junction toward the line of closure, where they join in a meshwork of anastomoses. Occasionally vascular twigs pass to the free margin of the leaflet. In some specimens vessels run upward in the chordae tendineae, stopping short of the free margin of the leaflet, many looping about to return to the papillary muscle. Occasionally, the chordae vessels form anastomoses with vessels in the basal third of the leaflet. Not infrequently, only one of the large descending vessels may be present, injection of the others having possibly failed through blockage with air, blood cells or a large carbon particle. The posterior mitral leaflet is supplied by three or more large vessels descending with loops, arborizations and anastomoses. These vessels lie close to the superior surface of

the leaflet In the non-cleared specimens they are not visible on the under side Histologically, the larger vessels show true arterial structure

Tricuspid leaflet injections are characterized by extremely fine vascular twigs extending downward 4 to 8 mm As in the mitral valve, the looping and interlacing arborizations are most impressive Vessels ending blindly indicate an incomplete injection

The pulmonary cusp vascularization, as a rule, is limited to the lower one-half or two-thirds of the membranous portion, with fine arterioles and capillaries extending into the cusps proper from along the line of attachment The filmy texture renders injection most difficult because of collapse or kinking of the delicate vessels

The capillary architecture of the aortic cusps is approximately the same as that of the pulmonary cusps, with a rich vascular bed in the commissures Vessels occasionally run along the line of closure to the corpus Arantii Studies are now in progress to determine whether the relatively small percentage of successful aortic cusp injections may be explained in many instances by the main blood supply being derived from the vasa vasorum rather than from the immediate coronary vessels

*Experiments on the Patency of the Blood Vessels of Nephritic Kidneys Obtained at Autopsy* By J M HAYMAN, JR, Philadelphia, Pa

One of the first questions which arise in the study of the physiology of the diseased kidney concerns the capacity of its vessels to permit a normal blood flow These experiments aimed to distinguish those types of kidney damage in which a mechanical, anatomic obstruction to blood flow exists from those in which the structural condition of the vessels might be regarded as permitting a normal blood flow

Kidneys obtained at autopsy were perfused with Ringer's solution at 100, 150 and 200 mm Hg pressure and the perfusate collected from the renal vein The results have been compared with roentgenograms of the arterial tree after injection of bismuth and with histological sections

Since large kidneys permit a greater volume of perfusate per minute than small ones, all data were reduced to flow per gram of kidney per minute This of course introduces a disturbing factor in kidneys edematous from disease, but this is offset by the advantages of the method in comparing perfusability of different kidneys

The volume of perfusate per gram of kidney in 18 normal adult kidneys varied from 1.2 to 2.5 cc per minute at 100 mm Hg perfusion pressure, from 2.1 to 4.3 cc per minute at 150, and from 2.5 to 5.5 at 200 mm pressure There was no detectable relation between volume of perfusate per gram of kidney and age

Kidneys showing purely degenerative changes, the so-called nephroses, allowed as great perfusion flows as normal kidneys This group includes a kidney of pregnancy and one of bichloride poisoning

Kidneys from 9 cases of benign arteriolarsclerosis showed a decrease in volume

of perfusate per gram roughly proportional to the degree of sclerosis. Two of these showed only mild changes histologically and the perfusion flows fell within lower normal range. Four kidneys showed moderately advanced changes histologically. One of these gave a perfusion flow within the lower normal limit, the other three distinctly below it. The other three kidneys showed advanced changes and the perfusion flow per gram of kidney was greatly reduced in all. Unfortunately suitable methods are not available for estimating quantitatively the decrease in number of patent glomeruli per gram so that it is uncertain what part decrease in number of perfused units and increased resistance in existing vessels play in the diminished volume of perfusate. Since the relative increase in volume of perfusate with increase in pressure is of the same order in normal and arteriosclerotic kidneys it seems probable that decrease in the number of patent glomeruli is the more important factor.

Two kidneys showing mild acute diffuse nephritis gave perfusion flows below the normal range. Kidneys from 5 cases of chronic glomerulonephritis and uremia showed a marked reduction of perfusate per gram of kidney at all pressures. The average flow per minute was 0.5 cc. at 100 mm. pressure, 0.9 at 150, and 1.2 at 200. These flows were of the same order as those found in advanced arteriosclerosis.

*Capillary Blood Flow in Man during Fever*<sup>1</sup> By FRANK FREMONT-SMITH, and (by invitation) L. RAYMOND MORRISON, and ALEXANDER W. MAKEPEACE, Boston, Mass.

Direct microscopic observations of the human nailfold capillaries have been made during the febrile reaction produced by the intravenous injection of typhoid vaccine.

Coincident with the onset of the fever there occurs complete stasis in practically every visible capillary. From a normal flow this stasis reaches its climax rapidly, often suddenly, and continues until the fever has nearly reached its height. During the period of stasis cyanosis occurs.

Just before the height of the fever is reached the blood flow in the capillaries begins again, at first slowly, but within a short time becomes enormously rapid with the appearance of capillary pulsation and the disappearance of the cyanosis. The fever now begins to fall. The rapid flow lasts throughout the period of falling temperature and often for many hours afterwards.

This same series of phenomena occurs in malaria and in sodoku (rat bite fever).

Evidence is brought forward to show that the stasis is produced by constriction of the terminal arterioles.

The local application of heat prevents the occurrence of the stasis, or abolishes the stasis once it has occurred.

At the meeting of this Society in April 1928 one of us (Fremont-Smith, F., Dailey M. E. and Thomas G. W.) presented evidence that dilution of the blood

<sup>1</sup> This investigation was aided by a grant from the Ella Sachs Plotz Foundation.

serum and cerebrospinal fluid occurs during the febrile response to intravenous injection of typhoid vaccine, analogous to the dilution seen in many acute infections

We have shown that this dilution of the blood serum occurs as a result of retention of ingested water within the organism. No dilution occurs if water is withheld. Water drinking normally results in prompt diuresis. During experimental typhoid vaccine fever, however, (and also in malarial fever) diuresis is delayed from six to twelve hours or longer.

The antidiuretic effect of typhoid vaccine fever begins coincidentally with the constriction of the skin arterioles described above. This could be explained by a similar vasoconstriction of the renal vessels. Mendelson, 1883, demonstrated such a vasoconstriction in the kidney during experimental fever in dogs.

The influence of this antidiuretic effect of fever upon the development of cardiac and nephritic edema is discussed.

*The Dynamics of the Circulation in Hypertension, and Its Bearing on Therapy* By  
SOMA WEISS and (by invitation) LAURENCE B. ELLIS, Boston, Mass.

Few, if any, systematic studies of the circulation in arterial hypertension have been made. As a result, there exists no adequate knowledge of the function of the circulation in this condition, and, therefore, rational therapeutic measures cannot be applied.

In an endeavor to throw some light on this subject, a group of patients with arterial hypertension, but without evident cardiac failure or kidney damage, were studied, with the object of discovering wherein the physiology of the circulation in hypertension differs from the normal. The arterial blood pressure was determined by the Riva Rocci method, the arteriolar and capillary blood pressure of the vessels of the skin was estimated with modified Recklinghausen capsules, the venous blood pressure was measured by the method of Moritz and Tabora. The vital capacity of the lungs was obtained with a portable spirometer, the cardiac output per minute and per stroke was ascertained by the carbon-dioxide method, the basal metabolism was obtained with the Tissot method. The velocity of blood flow was tested with the radio-active deposit and the histamine methods, and, finally, the blood volume was determined by the dye method. In a group of 16 patients, nearly all of the above measurements were undertaken repeatedly under basal conditions. In an additional group of 30 patients several of the circulatory functions were studied.

These findings indicate that, in essential arterial hypertension, although an abnormally high pressure exists in the arteries and arterioles, the capillary and venous pressures are normal. In other words, there is an abnormally great drop in the pressure gradient between the arterioles and capillaries. Notwithstanding the high pressure in the arteries, the average cardiac output per minute, the stroke volume of the heart, the velocity of blood flow, and the circulating blood volume are slightly less than in healthy individuals.

The resistance, which has been demonstrated to exist in the arteriolar portion of the vascular circuit, offers a considerable impediment to the cardiac function and markedly increases cardiac work. The development of the high pressure in the arteries may well be a compensatory response on the part of the circulation to reestablish the normal capillary blood flow and nutrition of the tissues.

The basal metabolism averages above the standard for normal persons. The efficiency of exchange of oxygen and carbon dioxide between tissues and capillary blood, judged from the ratio of tissue consumption of oxygen and the corresponding volume of blood flow, is better than in healthy people.

This study indicates that in order to gain beneficial results by means of a reduction of high blood pressure, the arteriolar resistance must be diminished. Therapeutic measures which induce a lowering of the blood pressure by lessening cardiac output or decreasing blood volume cannot be of permanent benefit.

*Cardiotachometric Studies of the Neurogenic Regulation of Ventricular Rate in Auricular Fibrillation* By ERNST P. BOAS, New York, N. Y.

The ventricular rate of patients with auricular fibrillation has been studied by means of the cardiotachometer. It has been shown that the ventricular rate is very variable, that it accelerates in response to the slightest exertion or emotion, and that it slows during rest and particularly during sleep. All of the evidence indicates that the ventricular rate in these patients is under control of the cardiac nerves, and that alterations in rate are governed by neurogenically determined changes in conductivity of the specific conducting tissue of the heart. The changes in ventricular rate arise, apparently, in response to the varying physiologic needs of the body, just as in health, but the reaction is not so well regulated and is often excessive.

Patients with auricular fibrillation may be classed in two groups—those with labile and with stable ventricular rates. The former are high strung and nervous and correspond to patients with neurocirculatory asthenia. Their ventricular rates tend to be rapid, and quantities of digitalis in excess of the body weight dose are required to keep the ventricular action slow and stable. In addition it would seem that sedative and psychotherapeutic treatment would assist materially in slowing the ventricles. In the stable group the ventricles do not exhibit such an exaggerated response to physical and emotional stimuli, and can be readily kept under control by the usual methods of digitalis therapy.

The value of rest and sleep in the treatment of patients with auricular fibrillation, a fact well known, is forcefully demonstrated by actual count of the number of heart beats by means of the cardiotachometer.

*Observations upon the Refractory Period of the Normally Beating Mammalian Heart*

By E. COWLES ANDRUS and EDWARD P. CARTER, Baltimore, Md.

Previous determinations of the refractory period of the mammalian auricle have involved driving the heart with a series of break induction shocks at a rate neces-

sarily more rapid than that of the spontaneous rhythm. The local stimulating effect of these repeated shocks has made accurate measurements possible only upon the atropinized heart, and in many instances, has led to false responses.

Using a specially designed apparatus the authors have determined the refractory period of the normally beating dog's heart. The action current is led off from the auricle, amplified to 12 to 20 volts, and applied to a light relay. Activation of the relay releases a pendulum which in turn throws in a single break induction shock at an interval controlled by the position of a tumble switch along its arc. By this means the normal excitatory process is utilized to control the timing of the interrupting shock. In contrast to earlier measurements the results of the authors show a sharp point above which each stimulus produces a response and below which excitation fails to occur.

A second difficulty in refractory period determinations in the past has been the necessity of introducing the interrupting shock at some distance from the recording electrodes on account of danger to the galvanometer string. This has been overcome by the use of a balanced circuit with a double induction coil as the source of stimulus and by grounding the proximal lead. With such a circuit it has been possible to place the stimulating electrodes actually astride the proximal lead.

A series of measurements has been made under a variety of conditions. The refractory period of the normal dog's auricle has been found to be between 0.08 and 0.12 second. Stimulation of the vagus brings about conspicuous shortening to 0.05 second or even less. A single dose of atropine sufficient to paralyze the vagus causes the refractory period to lengthen. Slow intravenous infusion of adrenalin produces considerable shortening but not to the degree noted under vagal stimulation. Moreover these changes in refractory period are not proportional to alterations in rate, in the case of vagus stimulation and paralysis, rate and refractory period vary in opposite directions.

It has been repeatedly observed that a stimulus introduced shortly after the end of the refractory period, during vagal stimulation, is followed not by a single response, but by auricular fibrillation. Such results have been reported by de Boer and others but always in hearts deprived of their blood supply or poisoned with various drugs and never under vagus stimulation alone. This suggests two points of interest. (1) Focus of stimulation—in every case the stimulus was applied well out upon the auricular appendix. (2) This rhythm followed stimuli which, in the absence of vagus stimulation, would have fallen within the refractory period. Due to shortening of the refractory period by the vagus they fell upon tissue which was excitable but in which recovery of conductivity had presumably not yet returned to normal. Hence as long as vagal stimulation was continued there existed in the auricular musculature, the conditions favorable to a re-entrant rhythm. The fact that under conditions of increased vagus tone, an extrasystole occurring early in diastole may lead to a re-entrant rhythm is, it seems, significant as a possible explanation of the genesis of auricular fibrillation.

*Orthopnea. Its Relation to the Increased Venous Pressure of Myocardial Failure*

By A CARLTON ERNSTENE (by invitation) and HERRMAN L. BLUMGART, Boston, Mass

The factors, which impel patients with myocardial failure of the congestive type to sit up in bed, have aroused the curiosity of many investigators. Insufficient attention, however, has been paid to the rôle of increased venous pressure in causing this phenomenon. The mechanism producing orthopnea in a patient with increased venous pressure due to uncomplicated congestive failure we believe to be as follows. Increased pressure within a vein causes slowing of blood flow in the corresponding capillary tributaries. If a patient with cardiovascular failure and a right auricular pressure equivalent to 15 cm. of water should lie flat in bed, the veins about the respiratory center would have a corresponding pressure of 15 cm. and the blood flow would be diminished. If, however, he should sit up so that the respiratory center were 15 cm. above the right auricle, the pressure within the veins leading from the respiratory center would be zero. The blood flow in the capillaries feeding these veins would then be increased, and the respiratory center might be expected to receive a more adequate blood supply. This should lessen subjective respiratory distress.

To test the validity of this theory, 82 comparisons of the height of venous pressure and the degree of orthopnea have been made in 21 patients with uncomplicated myocardial failure. The venous pressure was measured by the direct venepuncture method of Moritz and Tabora, and the degree of orthopnea was estimated by measuring the perpendicular distance between the right auricle and a point corresponding to the respiratory center—the external occipital protuberance. A parallelism between these measurements was, in general, observed—the higher the venous pressure, the greater the orthopnea.

Moreover, with the patient flat in bed, simple elevation of the head by flexing it on the thorax conspicuously diminished the respiratory distress in practically every instance. No other theory of orthopnea accounts for this phenomenon.

These and other observations indicate that, while other mechanical factors are not without importance, in uncomplicated myocardial failure the orthopneic position reduces subjective discomfort by releasing the respiratory center from the effects of increased venous pressure. This mechanism we believe to be of fundamental importance in the pathogenesis of orthopnea.

*Experimental Edema* By LOUIS LEITER (by invitation) and FRANKLIN C MCLEAN, Chicago, Ill

The well known association of low plasma proteins with certain forms of clinical edema and the concept of Starling that the osmotic pressure of the plasma proteins determines the reabsorption of tissue fluids into the capillaries, suggested the production of experimental chronic hypoproteinemia.

Dogs were bled 400 to 500 cc. twice daily from the heart and a corresponding volume of erythrocytes suspended in an alkaline Locke's solution was reinjected

intravenously Fifteen hundred cubic centimeters of 0.85 sodium chloride solution were given daily by stomach tube The concentration of plasma proteins was determined by the usual micro-Kjeldahl methods

Definite subcutaneous edema with gain in weight appeared by the fifth day in all of the dogs whose plasma protein level fell to about 3 per cent or less Ascites usually occurred at about the same time The edema ordinarily involved the external genitals, thighs, buttocks, perineum, and lower abdomen Discontinuance of bleeding for twenty-four hours led to prompt rise of the plasma proteins and rapid disappearance of edema in spite of continued administration of saline The entire cycle could be repeated on further plasmaphoresis

The subcutaneous edema and ascitic fluids resembled nephrotic edema fluid in appearance, with extremely low protein content—usually less than 0.1 per cent in the former and about 0.25 per cent in the latter This composition definitely ruled out the increased capillary permeability which is the cause of other types of experimental edema

Control experiments were made to rule out cardiac damage, under-nutrition, and high salt and water intake *per se*, all with negative results Plasma protein depletion alone does not lead to edema, hence, as in clinical edemas, the salt intake is an important factor This experimental edema was apparently on an extrarenal and mechanical basis analogous to that seen in some forms of Bright's disease and in undernutrition (war edema and cachectic edema)

*The Effect of Edema on Oxygen Utilization* By T. R. HARRISON and (by invitation) COBB PILCHER, Nashville, Tenn

The oxygen content of arterial blood and of blood from the femoral veins has been studied in patients with heart disease and in individuals without circulatory disorders The utilization (arteriovenous difference) is less in patients with cardiac edema than in the control group Average values for numerous analyses were

*Arterio-venous difference in volumes per cent*

| Cardiac patients |                |              |          | Normal controls | Non-cardiac edema |
|------------------|----------------|--------------|----------|-----------------|-------------------|
| Marked edema     | Moderate edema | Slight edema | No edema |                 |                   |
| 4.08             | 5.15           | 5.44         | 6.52     | 7.73            | 4.22              |

There were two exceptions to these general findings In patients with (a) digitalis intoxication or (b) acidosis, the utilization was high, whether edema was present or not

The low utilization in patients with edema was noted whether they were gaining or losing edema These observations are interpreted as follows Since there is hyperoxemia of the femoral venous blood in patients with edema of the legs, increased blood flow through the edematous legs seems probable This may be due to local oxygen lack in the tissues if, as seems likely, edema in the tissues, as in the

lungs, causes a partial obstruction to the passage of oxygen through the capillary wall. Hence, the oxygen pressure may be raised in the blood and lowered in the tissues. It seems that edema *per se* throws an additional strain on the heart by causing additional circulation to the edematous tissues and hence, that a vicious cycle may ensue. Therefore it is probable that the cardiac patient suffers not so much from cardiac insufficiency as from circulatory inefficiency.

It should be emphasized that the results found seem to be primarily effects, and only secondarily causes of edema.

*A Study of Serum Inorganic Sulfates in Renal Insufficiency* By E. G. WAKEFIELD  
(by invitation) and N. M. KEITH, Rochester, Minn.

*Further Studies on Deposit Nitrogen* By G. P. GRABFIELD, Boston, Mass.

In the attempt to apply previous studies on the mobilization and excretion of nitrogen in the body by means of iodides and salicylates to patients with chronic Bright's disease, it was found that patients showing the nephrosis syndrome excreted large amounts of nitrogen in proportion to the sulphur excretion. In comparing cases of Bright's disease without edema with those presenting the nephrosis syndrome, it was found that the former excreted urine, presenting the normal nitrogen-sulphur ratio and that these patients tended to exhibit negative sulphur balance. Patients presenting the nephrosis syndrome, on the contrary, showed a strong tendency to retain sulphur as compared with their retention of nitrogen. Such patients retained as much as 85 per cent of the sulphur of the diet. In a general way, of the patients presenting the nephrosis syndrome those that retained on high protein diets most sulphur in relation to the retention of nitrogen, showed the best clinical results in loss of edema and sense of well being.

These patients were kept for long periods on diets constant as to nitrogen, sulphur and phosphorus. The water intake was controlled and kept at a definite level by calculating the water content of the food and adding sufficient fluid to the diet to make a constant volume.

It was found that many of these cases were mixed and that some approached on lower protein diets the ideal condition in relation to nitrogen and sulphur retained.

The basis for further study of the sulphur metabolism in Bright's disease has been laid by these experiments, and it seems not unlikely that this component of the protein molecule will prove to be a factor of greater importance than it has hitherto been considered to be.

*Liver Fractions in Pernicious Anemia* By RANDOLPH WEST, and (by invitation) MARION HOWE, New York, N. Y.

We have shown that the substance in liver effective in pernicious anemia is precipitable by phosphotungstic acid but not by silver even in alkaline solution. The process now used is as follows:

Four hundred grams of Lilly Liver Extract is dissolved in 600 cc water and to this 200 grams anhydrous  $\text{Na}_2\text{SO}_4$  are added. After filtering off the precipitate which forms, sufficient 95 per cent alcohol is added to the filtrate to give a final concentration of 60 per cent. The supernatant alcoholic fluid is poured off within a few minutes after adding the alcohol and filtered. Alcohol is blown off from the filtrate with a hair dryer till volume is about 500 cc. The Kossel silver fractionation is then carried out using silver lactate and baryta, and excess silver removed from the filtrate as sulphide or chloride and baryta as sulphate. To the silver filtrate 3 per cent by volume  $\text{H}_2\text{SO}_4$  is added, and then a moderate excess of phosphotungstic acid (Kahlbaum). The phosphotungstic precipitate is dissolved in 75 per cent acetone and decomposed with hot aqueous baryta and filtered at the pump. Excess baryta is removed as sulphate, acetone blown off, and the material is ready for feeding. Yield about 3 to 4 grams solids. This fraction has never failed to give positive results on feeding in pernicious anemia. The best preparation fed to a case of tropical sprue in doses of 0.5 gram daily for eight days gave a reticulocyte peak of 200,000 per cubic millimeter, red blood cells being about 1.9 million. The poorest preparation fed in doses of 1.5 gram daily to a case of pernicious anemia gave a reticulocyte peak of 360,000 per cubic millimeter with red blood cells at 1.2 million. This is known as fraction B. It gives positive biuret, indole and diazo reactions, also a positive "arginine" reaction with a naphthol and sodium hypochlorite.

On treating fraction "B" with mercuric acetate at pH 6.0 practically all material precipitable with phosphotungstic acid is removed from the solution, but on regeneration of the precipitated bases with  $\text{H}_2\text{S}$  and feeding in 0.6 gram doses daily we have had negative results (one case tropical sprue with prompt response later) to 12 grams Lilly Extract daily.

On adjusting fraction B to pH 7.0, and adjusting volume so that the material derived from 800 grams Lilly Extract was in 100 cc water, and pouring this into 900 cc of acetone, the biuret positive material was largely precipitated. A case of pernicious anemia was given 1.5 gram of this precipitate, derived from several kilograms of Lilly Extract, daily for eight days with a negative result, and later responded well to liver. The acetone soluble material, after blowing off acetone, gave a strong diazo and indole reaction and an extremely faint biuret. This in doses of 0.6 and 0.8 gram daily has given weak positives (reticulocyte peaks of about 140,000 per cubic millimeters with red blood cells below 2.0 millions) in two cases, and a negative in one case.

On evaporating fraction B to small volume and making up to 100 cc (material from 400 grams Lilly) with absolute alcohol to give final alcoholic concentration of 74 per cent and treating with an excess of alcoholic platinum chloride a precipitate formed which on regeneration with  $\text{H}_2\text{S}$  and reprecipitation with platinum chloride was crystalline. The platinum content and the melting point of the aurichloride and mercurichloride agree with those of choline. Mixed melting point with known choline aurichloride showed no depression. An insoluble iodide,

resembling choline iodide in crystal form was also observed. On feeding this base in doses of about 700 mgm. daily for ten days to a case of pernicious anemia with red blood cells at one million per cubic millimeter the reticulocytes rose from 4.2 to 11.4 per cent. This material had been through platinum but once, and was derived from 4 kgm. of Lilly Extract. The effect was probably due to impurities accompanying choline. A second case fed 120 mgm. daily of material, twice through platinum, showed on the eleventh day of feeding a rise of reticulocytes, from 1 to 7 per cent with red blood cells at 1.0 million.

The platinum mother liquor, after removing Pt with  $H_2S$  on feeding 5.5 grams in one dose gave a reticulocyte peak of 150,000 per cubic millimeter with red blood cells at 1.7 million.

Synthetic choline, on feeding six grams in three days gave no reticulocyte response on the seven days following, whereas feeding 72 grams Lilly Extract in two days gave a reticulocyte peak of 300,000 per cubic millimeter with red blood cells at 1.2 million on the seventh day after feeding.

Fraction B has retained its full activity after removing all material precipitable with picric acid (4 grams solids in 20 cc. solution saturated with solid picric acid) and after shaking with butyl alcohol in both acid and alkaline solution.

*Serum Iron Studies* By HERMAN H. RIECKER and MARY E. WINTERS (by invitation) and HENRY FIELD, JR., Ann Arbor, Mich.

The object of this study was to determine whether or not a relationship existed between (1) the level of serum iron, hemoglobin production, and iron administration in the experimental anemia of hemorrhage, (2) the level of serum iron and the iron metabolism in pernicious anemia during active remission, and (3) the serum iron content and the hemoglobin percentage in various secondary anemias.

Iron determinations were made by Elvehjem's and Hart's modification of the Thompson method. The element of error possible from hemolysis was avoided by precipitation of the serum proteins with trichloroacetic acid, following the suggestion of Briggs.

The animal experiments were conducted upon dogs by a method essentially similar to that devised by Whipple and Robschert Robbins.

The following relations were noted. As the anemia progressed there occurred a depression in the serum iron level from the normal of about 1.2 mgm. per 100 cc. of blood to between 0.7 and 0.9 mgm. during which time the animal did not regenerate hemoglobin. When Fe was added to the diet in any form regeneration began and the serum iron level definitely rose.

Then a series of six pernicious anemia cases were studied during relapse and remission. Blood serum iron determinations were made and the intake and excretion of iron were determined. The serum iron figures were all above, or within, the normal limits.

The iron excretion of patients before treatment on a constant intake of 7 to 15 mgm. was found to be fairly constant, as was the control case. However, when

treatment was begun with liver extract a marked increase of excretion over intake occurred and continued throughout the periods of observation

In the secondary anemias those due to hemorrhage were separated from those of an unidentified nature. In cases with acute hemorrhage the serum iron levels were in general lower than in the other types. The studies in secondary anemia have not been completed so that the results given merely indicate the trend of the investigation

*Conclusions* 1 In the experimental anemia of hemorrhage a definite iron starvation is present and this is relieved by iron feedings with a rapid increase in hemoglobin production. Serum iron determination may be used to detect this condition

2 In pernicious anemia there is a well marked negative iron balance during the remission induced by liver extract. The serum iron levels tend to be increased above the average normal in relapse, and to approach the normal in remission

3 No degree of iron starvation could be found in secondary anemias comparable to that in experimental animals with anemia due to hemorrhage

*The Endogenous Uric Acid Metabolism in Pernicious Anemia* MATTHEW C RIDDLE, (by invitation) and CYRUS C STURGIS, Ann Arbor, Mich

The endogenous uric acid metabolism was studied during early remission in twelve patients with pernicious anemia, using purine-poor diets over periods of 10 to 75 days

Before treatment, in two patients the concentration of uric acid in the blood serum was higher than normal, in three it was normal, and in six lower than normal. Only one patient excreted increased amounts of uric acid before treatment

During remissions induced by liver extract therapy there was an increase in the urinary excretion of uric acid of from 74 to 531 per cent and an increase in concentration of uric acid in the blood serum of from 28 to 239 per cent which was correlated with the rise in percentage of reticulocytes. The increased uric acid metabolism was shown to be endogenous in nature by the failure of impotent liver extract to produce the typical results, by the typical uric acid increase both following the administration of a single massive dose of liver extract and during spontaneous remission and by the failure of liver extract to increase the uric acid metabolism in a normal person

The increased uric acid metabolism may be associated with the increased rate of destruction of red blood cell nuclei and a general increase in nuclear metabolism during remission

*Calcium Metabolism in Hyper- and Hypo-Parathyroidism* By H A BULGER and D P BARR, St Louis, Mo

Recent advances in our knowledge of calcium metabolism, especially those afforded by Collip's parathyroid extract, have placed us in a position to recognize the clinical picture of hyperparathyroidism. Extensive study of two such patients

has revealed typical symptoms and associations, together with characteristic abnormalities of calcium metabolism. More calcium than ingested was excreted by the intestines alone, although there were also excessive amounts in the urine. There was a negative phosphorus balance.

A great reduction in calcium in the urine and a positive calcium balance could be obtained by administration of phosphates.

These abnormalities were compared with and were in striking contrast to those found in hypoparathyroidism, in which there have been almost negligible amounts of calcium in the urine and a remarkable tendency to retain calcium.

One patient with hyperparathyroidism was studied in all stages of transition to extreme hypoparathyroidism which followed the removal of a parathyroid tumor.

*The Specific Dynamic Action of Protein in the Obese* By E. F. DU BOIS and (by invitation) H. J. SPENCER, W. S. MCCLELLAN and E. FALK, New York, N. Y.

One explanation of obesity is that the specific dynamic action of food stuffs is below normal. There is considerable doubt, judging from the literature, whether this is a fact. We have studied the influence of protein in obese and normal individuals by means of the respiration calorimeter.

Twelve observations following meals of 300 to 500 grams of lean meat were made on 4 obese and 3 normal men of various ages who were studied for considerable periods in the metabolism ward. These observations show considerable variation in the heat output from hour to hour both in the individual records and in the group averages. The extra heat production, expressed as percentages of the basal heat output, has been calculated hourly from the time the meat was ingested.

The average increase in caloric output of the obese men surpassed the normal in the fourth hour. The normal men put out heat more uniformly. The average surplus heat output per hour was approximately 13 per cent for the obese, and 17 per cent for the normal men.

Our impression is that, while the obese man responds to protein more irregularly than the normal man, his extra heat output is not significantly different from the normal.

*The Effect of Lessened Respiratory Reserve on the Blood and the Circulation: An Experimental Study* By WILLIS S. LEMON, Rochester, Minn.

A series of experiments was carried out with the object of observing the effect of lessened mechanical efficiency on respiratory reserve. It was hoped also to discover the point at which reserve is lost, respiration as a function becomes inadequate and circulatory aid is required to maintain comfortable existence. The animal studied was an intelligent dog which had previously been taught to lie quietly while wearing the mask usually employed for basal metabolic estimations.

At approximately weekly intervals during March and April of 1927, the dog was subjected to four operations carried out under ether anesthesia and aseptic tech-

nic These operations were (1) evulsion of all the left intercostal nerves, (2) evulsion of the lower eight right intercostal nerves, (3) evulsion of the right phrenic nerve, and (4) evulsion of the left phrenic nerve

As a result of this procedure, respiration depended on the actions of the muscles attached to the ribs posterior to the operative sites, and those extra-respiratory muscles about the neck and shoulders which are used normally only following extreme muscular exertion. The respiratory function of the diaphragm was of course excluded, and its effect on intraabdominal pressure removed, its only rôle was that of a partition separating thorax from abdomen

The behavior of the dog following surgical interference was in every respect similar to that observed in a previously reported group of experiments. As a result of section of the intercostal nerves on one side, there was immediate or delayed, temporary or persistent, asymmetry of the chest, with a definite and measurable loss in outward movement of the costal arch and margin during inspiration. The reduction amounts to from 30 to 50 per cent of normal, depending on the number of nerves cut. When half the diaphragm is paralyzed, no outward movement of the costal arch margin occurs during inspiration. Symmetric reduction of movement results from bilateral section of the intercostal nerves, and regardless of the order in which phrenic nerve paralysis is introduced, the movements remain the same. The animal of the present experiment after operation lived comfortably, played about like its normal mates and on casual examination could not be distinguished from them. It could not, however, respond to vigorous exercise with the same ease owing to the limitation of vital capacity.

In accordance with the object of this investigation, the intake of air for each unit of time was measured both before and after the series of operations, since it was believed the results would be equivalent in value to alterations in volume. The figures indicated that the respiratory function was adequate to supply sufficient ventilation under all conditions and that the vital capacity provided for a normal amount of tidal air.

The animal was returned to the kennels and apparently lived a normal life during the following year. Despite the paralysis of the whole diaphragm and upper abdominal muscles, difficulty in urination or in defecation was not observed. The lower abdominal muscles, however, had undergone hypertrophy.

Exactly one year after the last operation, the abdomen was opened. The diaphragm was found to be high in the thorax and so thinned that the lungs were plainly visible through it. Specimens from each leaf of the diaphragm were removed for study. After the animal had recovered from the operation, it was observed that as the chest was raised during inspiration, the lower abdominal muscles contracted strongly. This suggests a compensatory attempt to retain the abdominal contents in the abdomen, thus preventing respiratory embarrassment.

Twenty months after the first operation, the blood was studied to determine whether or not the long continued reduction in vital capacity could have brought

about erythrocytosis, changes in blood volume, in hematocrit values, in hemoglobin, in oxygen, in carbon dioxide, or in the blood count. Electrocardiographic tracings were also made. Repeated calculations and comparisons with values obtained in normal dogs did not reveal significant alterations in the experimental animal. This confirms the opinion that the animal retained sufficient respiratory reserve and under ordinary living conditions did not require assistance from the circulation.

At the end of February, 1929, a second laparotomy was performed. After the adhesions were separated, it was found that the stomach, part of the duodenum and all but two of the lobes of the liver had migrated up into the thorax, this confirmed previous roentgen ray data. The part of the diaphragm which yet remained was, as previously described, thin and translucent and flapped back and forth with respiratory movements. An incision through its costal attachments was made on each side thus eliminating its action as even a partition. The abdomen was then closed and on recovery from the anesthetic the dog ran about the room without any apparent respiratory embarrassment. A regimen of rest was imposed to encourage healing and particularly to allow development of compensation and to avoid unnecessary strain.

Examinations of the blood, March 8 and 16, showed, respectively, hemoglobin 85 and 90 per cent, erythrocytes 4,910,000 and 5,760,000 and leukocytes 15,900 and 12,400 for each cubic millimeter. The differential count was essentially normal as also was the percentage volume of erythrocytes. The air intake each minute was somewhat larger than previously, being 4.56 liters as compared with 3.18 preoperatively. The respiratory rate each minute had increased to 22 from a preoperative average of 12. The pulse rate remained the same. Hematocrit varied from 42 to 45 per cent of cells. Blood volume was 86 cc per kilogram. The electrocardiogram was normal.

*Summary* Vital capacity has been lessened, but never beyond being equivalent to tidal air, ventilation volume was raised somewhat, but never to an abnormal degree. The hemoglobin remained within the normal limits as also did the hematocrit percentages. Although the oxygen saturation of both arterial and venous blood was lowered the arterial and venous oxygen content was essentially normal. There was no erythrocytosis and the heart showed no evidence of abnormality.

These findings would seem to confirm the independence of the fundamental functions of the cardiac and respiratory systems, great loss of reserve in one, being necessary before demonstrable evidence of coöperative support is provided by the other.

*The Experimental Production of Chronic Abscess of the Lung* By LOUIS G. HERRMANN, (by invitation) and ELLIOTT C. CUTLER, Cleveland, Ohio

A study of the factors that are responsible for the chronicity of the suppurative processes of the lung was made. The marked increase in the intrabronchial pressure associated with constant violent cough has been shown to play a minor

rôle in the prolongation of the activity of these pulmonary lesions. In the experiments previously reported from this laboratory only aerobic microorganisms were implanted in the pulmonary tissue by means of the vein-segment embolus. The lesions that resulted were all of the acute type and they healed spontaneously within three weeks.

Recently Smith showed that the bacteria present in chronic abscess of the lung in human beings were morphologically identical with the bacteria present in the material from the margins of the gums of patients with pyorrhoea alveolaris.

A detailed study of this material was made and the embolic method of implantation of this material into the pulmonary tissue was utilized in a series of 38 dogs. With one exception each animal developed a localized abscess at the site of lodgment of the embolus. Of the 38 dogs 28.9 per cent developed pulmonary abscesses which remained active for more than five weeks. In two of the animals the lesions remained active for 101 days and 126 days respectively. At autopsy the cavities of the abscesses were filled with grayish-green necrotic material that had a very foul odor. There was a thick wall of induration about the cavity of the abscesses.

The material from these experimentally produced chronic pulmonary lesions was cultured on a great variety of culture media and under both aerobic and anaerobic conditions. In all, twelve different varieties of microorganisms were isolated in pure culture,—five aerobic microorganisms and seven anaerobic microorganisms.

These pure cultures of microorganisms were then implanted into the pulmonary tissue of another series of dogs. The aerobic organisms produced only acute lesions that healed spontaneously within three weeks. The lesions that resulted from the implantation of the anaerobic microorganisms in the pulmonary tissue were of longer duration and several of them remained active for more than five weeks. Pure cultures of the *Spirochaeta microdentium* and the large and small variety of fusiform bacilli were also implanted in the pulmonary tissue but no lesion developed at the site of lodgment of the embolus.

Several combinations of these anaerobic microorganisms were used and the lesions that were produced more closely resembled the true chronic abscesses of the lung which are found in man.

Our studies show that the anaerobic group of organisms are the important factors that determine the chronicity of pulmonary lesions. Further experiments are being conducted with the hope of determining the exact combination of anaerobic microorganisms that is responsible for the chronicity of these lesions.

*The Flora of the Upper Respiratory Tract of Infants in the First Year of Life*. By YALE KNEELAND, JR., (by invitation) and A. R. DOCHEZ, New York, N. Y.

In connection with studies on the common cold, it was thought advisable to investigate the upper respiratory flora of infants. This report is based on data derived from thirty normally-delivered infants in Sloane Hospital, a number of

these were subsequently followed in the out-patient department, and observations were also made on infants of the same age with and without colds. As a rule the upper respiratory tract is sterile at birth, no growth being obtained in 85 per cent of the nasal cultures, and 80 per cent of the throats in the first day of life. After the first feeding however, bacteria are regularly found in the throat, although the nose may remain sterile for another 24 hours. Growth from the nose is rarely abundant the *Staphylococcus albus* being the most prominent organism. Diphtheroid bacilli rank next, with *Staphylococcus aureus* and large Gram positive cocci following. Streptococci are relatively less frequent, occurring in but 19 per cent of all cultures. Members of the colon group and Gram negative cocci are rarely encountered. From the throat growth is usually abundant with streptococci the outstanding organism, the indifferent (gamma) type being more frequent than the green producing varieties. Staphylococci are also very common. Colon bacilli are occasionally seen, while diphtheroid bacilli and Gram negative cocci are rare. In the first two weeks of life, *B. Pfeifferi*, pneumococcus and hemolytic streptococcus were never encountered either in the nose or throat.

For comparison with this group, twenty eight normal infants from four to eight months of age were studied, and certain differences noted. In the nose the staphylococci were less outstanding, with a corresponding rise in the Gram negative cocci (18 per cent as opposed to 1 per cent in the new born), and the appearance of *B. Pfeifferi* and pneumococci in 7 and 14 per cent of the cases respectively. The throat showed a marked increase in the green streptococci, which appeared in 100 per cent of cases, at the expense of indifferent streptococci and staphylococci. The incidence of Gram negative cocci went from 4 per cent to 71 per cent, and *B. Pfeifferi* was found in 53 per cent of cases. Hemolytic streptococci appeared in 11 per cent. (Nineteen of these normal infants gave a history of having had colds and nine did not, there was no difference between the two save for a somewhat higher incidence of Gram negative cocci in the throats of the former.)

Observations were also made on twenty-one infants early in the course of their first colds, the average age being three months. There was no essential difference between their flora and that of the normal group, with the exception that they showed a higher incidence of diphtheroid bacilli, and Pfeiffer less frequently. From a clinical standpoint, these first colds are mild, characterized by a moderate degree of coughing and sneezing, with at times a little coryza, usually without constitutional symptoms.

Lastly, a group of fourteen infants with recurrent colds were studied, the average age being seven months. One striking change was noted from the normal controls, namely the increase of *B. Pfeifferi* and pneumococci in the nasal cultures to 21 and 43 per cent respectively. The latter was actually predominant in 36 per cent of cases. It may be added that many of these children had a purulent nasal discharge, and sinusitis was not improbable. The flora of the throat remained unchanged.

From the evidence thus far obtained, it would appear unlikely that there is any

specific bacterial incitant of the early colds of infancy, although in the chronic recurrent type of cold bacteria probably play a part. On the other hand, such pathogens as *B. Pfeifferi*, pneumococcus, and hemolytic streptococcus may occur in normal infants without giving rise to symptoms.

*Chloride Balance in Pneumonia*<sup>1</sup> By J. H. AUSTIN and (by invitation) F. W. SUNDERMAN, Philadelphia, Pa.

The intake and output of chloride have been measured in seven patients with lobar pneumonia. Four of these patients received the usual diet and therapy, three received large amounts of sodium chloride (8 to 30 grams per day) by mouth during the precritical period. In the first group although during the precritical period excretion of chloride in the urine was very low nevertheless the total excretion of chloride exceeded the intake. In the second group the excretion of chloride in the urine was increased but was less than in normal subjects. In the second group there was consistently retention of chloride before the crisis. Our studies support the view that lobar pneumonia is characterized during the precritical period by a diminished capacity to conserve chloride on a low intake of chloride and a diminished capacity to excrete chloride on a high intake of chloride.

*The Experimental Reproduction of the Blood Picture of Infectious Mononucleosis in the Guinea Pig* By L. W. GORHAM and (by invitation) DAVID T. SMITH and H. D. HUNT, Albany, N. Y.

Infectious mononucleosis is a benign sporadic disease occurring in young adults, and is characterized by fever, sore throat, and enlargement of the glands and spleen. Although Turck studied a case in 1907 the real interest in this disease dates from the contribution of Sprunt and Evans in 1920.

The frequent occurrence of sore throat in these cases suggested that the infectious agent either remained localized to the pharynx or entered the body through the pharynx. Some of the cases present typical Vincent's angina of the pharynx, while others do not.

The first case studied was a typical case of infectious mononucleosis in a young girl who had a very severe Vincent's angina of the tonsils. Some of the membrane from the pharynx was inoculated into the groin of two guinea pigs and both animals developed the typical blood picture of infectious mononucleosis. Dr. H. D. Hunt developed infectious mononucleosis subsequent to his contacts with the patient and with the infected guinea pigs. The mother of Dr. Hunt also developed the disease apparently as the result of direct contact. Material removed from the throat of Dr. Hunt with a suction apparatus produced the blood picture of the disease in the guinea pigs. Later in the experiments Miss R., who was counting the blood of the infected guinea pig, developed infectious mononucleosis. Material removed from her throat also produced the typical blood picture in

---

<sup>1</sup> This investigation was aided by a grant from the Ella Sachs Plotz Foundation.

guinea pigs. So the blood picture of the disease has been reproduced in guinea pigs with material from the pharynx of three different cases. One of these had a typical Vincent's infection of the throat, one had a typical Vincent's infection but the throat did not have Vincent's infection.

An examination of Dr. H. D. Hunt's throat in the early stage of the disease revealed a very large number of vibrios. Since very little is known of the blood reaction to vibrios we decided to isolate and study this organism first. Two strains were isolated, one from a case of Vincent's angina and one from a case of acute Vincent's infection of the bronchi. Both these strains produced typical blood pictures in the guinea pig. One of these strains was used only as a dead heat-killed vaccine. This dead material produced just as typical changes in the blood as did the living organism of the other strain. After recovering from one attack the animals were immune to a second or third inoculation. There was cross immunity between the two strains. Blood from Dr. Hunt agglutinated a vibrio from a different source in a dilution of 1:64. Two cubic centimeters of this serum prevented the appearance of the blood changes in the guinea pig for one week, after which time the typical changes appeared. A rabbit was immunized to the vibrio and its blood agglutinated the organism in a dilution of 1:320. Five cubic centimeters of this serum prevented the development of the blood changes in a guinea pig inoculated with 2 cc. of a vibrio culture. Five cubic centimeters of blood from a normal rabbit failed to protect a guinea pig inoculated with the same amount of same culture.

Cultures of (1) fusiform bacilli, (2) *Streptothrix*, (3) *Staphylococcus albus*, (4) *Staphylococcus aureus*, (5) *Streptococcus hemolyticus*, (6) *Streptococcus viridans*, (7) *Bacillus coli*, and (8) diphtheroid all failed to produce this typical picture.

*The Effects of Over Dosage with Irradiated Ergosterol* By A. T. SHOHL and (by invitation) HARRY GOLDBLATT, Cleveland, Ohio

Rats were fed normal diets and also ricketogenic diets of both the high calcium and low phosphorus type and high phosphorus and low calcium type. Four mgm. of irradiated ergosterol (Vigantol) were administered daily. They died approximately in seven days. Marked calcification of the blood vessels of the heart, kidneys and stomach was observed and hypercalcification of the epiphyses of the long bones. Calcium and phosphorus of the blood serum and the ash of the fat-free bones were determined. Control experiments with unirradiated ergosterol or olive oil showed none of these abnormalities.

*Studies on the Value of Acid Neutralization in the Treatment of Gastrointestinal Ulcer*  
By JOSEPH A. CAPPS and (by invitation) WALTER LINCOLN PALMER, Chicago, Ill.

In this paper a comparison is made between two similar groups of patients, each comprising 27 cases of gastric and duodenal ulcer treated under the same conditions as regards rest and gastric evacuations, with the same diet and with

hourly feedings of milk and cream Alkaline powders dissolved in 90 cc of water were administered hourly in one group in doses large enough to neutralize the gastric free acidity as completely as possible throughout the entire digestive period, in the other group, 90 cc of 10 per cent Liebig's beef extract was given every hour in place of the powders, the idea being to stimulate gastric secretion thereby The duration of spontaneous pain was observed in each group and every fourth day an "acid test" performed for the purpose of ascertaining the duration of sensitivity to this test The results are based upon this comparison The alkali group showed a much more prompt cessation of spontaneous pain than did the beef tea group and became acid insensitive more quickly

*A Study of the Reflex Influence of the Colon on the Stomach* By FRED M SMITH, and (by invitation) GEORGE H MILLER, Iowa City, Iowa.

There is a considerable difference of opinion relative to the possible reflex influence of the colon on the stomach Epigastric pain, nausea, and vomiting are frequently associated with chronic colitis Clinicians and roentgenologists have been inclined to attribute these symptoms to a disturbed motor function of the stomach induced by a reflex stimulation from the colon The experimental investigation of this problem, however, has led to varying conclusions

In the present investigation, dogs, anaesthetized by barbital were employed A mid line incision was made in the upper abdomen which would permit exposure of the stomach and proximal colon The stomach was filled with warm water and the activity observed during a control period Croton oil was then introduced into the proximal colon Precautions were taken to prevent the irritant coming in contact with the ileum In some instances the bowel was later distended with air Following the introduction of the irritant into the colon there was usually a marked increase in the activity of the stomach The peristaltic waves were much more frequent, deeper, and at times very violent During the height of the increased activity, reverse waves were occasionally noted The activity of the stomach was often further intensified by distending the colon In experiments in which the irritation was confined to the appendix, the effect on the stomach was similar The activity of the stomach induced by the stimulation of the colon or appendix was abolished by atropin

*The Systemic Effect of the Presence of Bile in the Peritoneal Cavity* By G O BROWN and (by invitation) A P BRIGGS, St Louis, Mo

Whole bile or solutions of bile salts introduced into the peritoneal cavity give rise to a severe serofibrinous peritonitis Vomiting and diarrhea occur leading to dehydration There is a marked fall in blood pressure and extreme prostration and weakness The heart rate may be slowed in some cases In others it becomes more rapid The electrocardiogram shows some irregular changes in the T-waves Secretion of urine is decreased or completely suppressed

Changes in blood chemistry are those expected with dehydration and decreased

urinary output. Blood chlorides show a moderate fall. Non protein nitrogen and inorganic phosphate are markedly increased. Plasma protein increased slightly. Changes in blood lipoids and blood sugar were irregular and not marked. Tests for the presence of bile salts in the blood stream gave positive reactions. In dogs no increase in blood bilirubin was found although bilirubinuria occurred.

Concentration of the blood is shown by decided increases in red count, cell hematocrit and hemoglobin content. A marked polymorphonuclear leucocytosis occurs. A moderate increase in the fragility of the red corpuscles is observed. Some free hemoglobin may be found in the blood plasma. In the absence of infection, erythrocyte sedimentation rate is delayed. Clotting time is slightly delayed but may remain within normal limits.

*The Respiratory Metabolism of the Tubercle Bacillus*<sup>1</sup> By R. O. LOEBEL, E. SHORR (by invitation) and H. B. RICHARDSON, New York, N. Y.

In the study of tuberculosis it is desirable to know the processes by which the parasite obtains energy and the influence of adverse conditions on its respiratory metabolism.

The following is a study of the oxygen consumption of the tubercle bacillus as influenced (1) by the age of the culture (2) by the constituents of the nutrient medium, and (3) by the pH of the solution.

After 8 days cultivation on Long's synthetic medium the consumption of oxygen, measured in the micro-respiration apparatus of Warburg, averaged 2.6 cmm. per milligram of moist weight, per hour, after 15 days, 2.2 after 22 days, 0.45, and after 29 days practically zero. The maximum was about twice as much as reported for mammalian leucocytes. It was about one fifth that of a rapidly growing organism of the acid fast group, i. e., the smegma bacillus.

In order to establish a base line for the study of nutrients the organisms were transferred from Long's medium to a similar solution containing only the salts of sodium and potassium. After 6 days the respiration became much reduced, but on retransfer to Long's medium regained nearly its original level. The oxygen consumption in Long's medium could be fractionated as follows:

|   | per cent |
|---|----------|
| Starvation level  | 6        |
| Effect of glycerol  | 73       |
| Effect of nitrogenous substances (asparagin and ammonium citrate) | 19       |
| Effect of ferric ammonium citrate and magnesium sulfate.          | 2        |

After starvation the organisms retained their original power of growth.

No increase in respiration above the starvation level was observed as the result of transfer from buffered saline to a similar solution containing 5 per cent glucose.

<sup>1</sup> Aided by a grant from the Research Committee of the National Research Association.

or levulose This is in accord with the well known difficulty of cultivating the organisms by the use of sugars Sodium lactate caused a rise which even exceeded the effect of glycerol, but was entirely ineffective as a substitute for glycerol in the promotion of growth This is an instance in which the energy metabolism is dissociated from growth

TABLE 1

*The influence of foodstuffs on the growth and respiration of the tubercle bacillus—II 37*

| Duration                            | Oxygen consumption per moist milligram |                     |                       |                    |                     | Remarks                        |
|-------------------------------------|--|---------------------|-----------------------|--------------------|---------------------|--------------------------------|
|                                     | No foodstuff                           | Glycerol 5 per cent | Na lactate 5 per cent | Glucose 5 per cent | Levulose 5 per cent |                                |
| <i>hours</i>                        | <i>c mm</i>                            | <i>c mm</i>         | <i>c mm</i>           | <i>c mm</i>        | <i>c mm</i>         |                                |
| 1                                   | 1 1                                    | 1 8                 | 2 1                   | 1 3                | 1 2                 | 16 d culture<br>2 d starvation |
| 1                                   | 0 6                                    | 1 5                 | 2 4                   | 0 9                | 0 5                 | 16 d culture<br>2 d starvation |
| 1                                   | 1 0                                    | 1 2                 | 1 6                   | 0 9                | 0 7                 | 15 d culture                   |
| 2                                   | 1 8                                    | 2 4                 | 3 3                   | 1 8                | 1 5                 | 2 d starvation                 |
| Growth on a modified Long's medium* |  |                     |                       |                    |                     |                                |
|                                     | None                                   | Profuse             | None                  | Slight             | None                |                                |

\* The foodstuff at the head of the column was substituted for the glycerol of Long's medium

In saline solutions buffered with phosphate mixtures the oxygen consumption for short periods was much the same over a range extending from pH 1.5 to 12.0

TABLE 2

*The influence of hydrogen ion concentration on respiration of the tubercle bacillus—II 37*

| Duration     | Oxygen consumption per moist milligram |             |             |             |             | Remarks—phosphate buffers M/150 |
|--------------|--|-------------|-------------|-------------|-------------|---------------------------------|
|              | pH 1.5                                 | pH 5.6      | pH 7.4      | pH 8.8      | pH 12.0     |                                 |
| <i>hours</i> | <i>c mm</i>                            | <i>c mm</i> | <i>c mm</i> | <i>c mm</i> | <i>c mm</i> |                                 |
| 1            | 1 0                                    | 1 0         | 1 1         | 1 1         | 1 2         | 16 d culture<br>3 d starvation  |
| 1            | 1 3                                    | 1 6         | 1 6         | 1 4         | 1 5         | 10 d culture                    |
| 2            | 2 3                                    | 3 3         | 2 8         | 2 6         | 2 8         |                                 |
| 3            | 3 3                                    | 5 1         | 4 4         | 4 0         | 4 3         |                                 |
| 1            |  | 1 7         | 1 8         | 1 5         |             | 10 d culture                    |

*The Chemical Metabolism of Normal and Diseased Lymph Nodes* By HENRY JACKSON JR., and (by invitation) FREDERIC PARKER JR., and E. C. GLOVER, Boston, Mass

For years there has been much discussion as to the nature of those diseases which may be classified under the general heading malignant lymphoma or lymphoblastoma. Some pathologists hold that they are true malignant processes, while others maintain that they are infectious or granulomatous in nature. Warburg has shown that with certain exceptions malignant disease tissue has a characteristic metabolism. In brief, neoplasms use but little oxygen but consume large quantities of sugar under both aerobic and anaerobic conditions, whereas normal organs destroy but little sugar either in the presence or absence of oxygen and embryonic material destroys but little sugar under aerobic but large amounts under anaerobic conditions.

We have examined the metabolism of twenty-two malignant lymphomas and compared these with the metabolism of normal and tuberculous nodes and with nodes containing metastatic malignancy. We have also included determinations on several benign tumors.

Our findings may be summarized as follows:

*Average glycolysis*  
(Milligrams per milligram hour)

|              | Aerobic | Anaerobic |
|--------------|---------|-----------|
| Normal nodes | 0.020   | 0.035     |
| Lymphomata   | 0.050   | 0.075     |
| Tuberculosis | 0.075   | 0.106     |
| Carcinoma    | 0.110   | 0.134     |

*Extremes of glycolysis*  
(Milligrams per milligram hour)

|              | Aerobic   | Anaerobic  |
|--------------|-----------|------------|
| Normal nodes | 0.00-0.05 | 0.01-0.075 |
| Lymphomata   | 0.02-0.10 | 0.04-0.140 |
| Tuberculosis | 0.05-0.13 | 0.08-0.19  |
| Carcinoma    | 0.07-0.17 | 0.09-0.23  |

In order to overcome certain exceptions and difficulties Warburg introduced a value 'U' which was the anaerobic glycolysis less twice the oxygen consumption. This value he found to be negative for normal, zero for embryonic and a high positive for cancerous tissues.

Calculating this figure from our data we find

*Value for "U"*

|               |      |
|---------------|------|
| Benign tumors | 2 1  |
| Normal nodes  | 4 3  |
| Lymphomata    | 10 0 |
| Tuberculosis  | 10 2 |
| Carcinoma     | 28 7 |

It is difficult to draw definite conclusions from these data at the present time. More work is being done and the data elaborated. For the present we would point out

1 There is considerable overlapping of the various classifications. By metabolic figures alone the tissue cannot be surely classified. The value U is more consistent.

2 From the value U one would place the malignant lymphomata among the granulomatous rather than the neoplastic diseases.

3 Further study is being made of the relation of the metabolic changes to the particular cell involved.

*The Effect of Iron on Blood Formation as Influenced by Changing the Acidity of the Gastric Contents in Certain Cases of Anemia* By STACY R. METTIER, (by invitation) and GEORGE R. MINOT, Boston, Mass.

The oral administration of iron produces in certain cases of "secondary" anemia a response of the reticulocytes. The anemia in the 4 cases reported upon was associated with defective diets and disorders of digestion or chronic blood loss. In these cases when the gastric contents was made neutral in reaction, the feeding of a given daily amount (0.08 to 0.32 gram) of iron produced a response of reticulocytes. Soon afterwards, however, when the same dose of iron was maintained, a second and greater response of these cells occurred after the gastric contents was rendered acid.

In the same case two distinct responses of the reticulocytes within a short period of time may be interpreted to indicate that the cause of the second response is more potent than that producing the first. Furthermore, during a third period when the amount of iron was increased four fold or more in these cases, there developed a third response of the reticulocytes. This indicated that iron was the essential factor in producing the response, modified by changes in the acidity of the gastric contents, and is distinct evidence that large doses of iron may be much more effective than relatively small ones. The patients with great rapidity increased to normal the concentration of their hemoglobin and red blood corpuscles.

*Urobilinogen Excretion in Addison's "Pernicious" Anemia Before and After Liver Therapy* By DUNCAN GRAHAM and (by invitation) RAY FARQUHARSON, HENRY BORSOOK, and A. M. GOULDING, Toronto.

In Addison's "pernicious" anemia in state of relapse with anemia the urobilino-

gen excretion is greatly increased and may be several times the normal value. This is usually associated with a high bilirubin content of the blood plasma. Following liver therapy and beginning about the peak of the reticulocyte response there is always a sharp decrease in urobilinogen excretion which falls in a few days to a point within normal limits. In severe cases the amount of urobilinogen excreted per day is equivalent to 9 to 12 grams of hemoglobin and in one case it amounted to one-eighth of the total blood hemoglobin of the body.

In some cases of Addison's "pernicious" anemia showing little anemia but presenting other manifestation of the disease such as subacute combined degeneration of the cord, the blood bilirubin is usually normal but the urobilinogen excretion is increased somewhat above a high normal value. On liver therapy such cases show a low reticulocyte response and the urobilinogen excretion falls slowly and gradually to within normal limits.

These observations on urobilinogen excretion in cases of Addison's "pernicious" anemia go to show (1) that in the state of relapse there is a markedly increased blood destruction, (2) that adequate liver treatment controls this destruction, (3) that the rapid fall in urobilinogen excretion, beginning during the reticulocyte response, suggests that the abnormal red blood corpuscles present in a relapse are the ones chiefly affected in the stage of increased blood destruction.

*Some Effects of Adrenalin on the Heart with Especial Reference to Its Use As a Test for Angina Pectoris* By S A LEVINE, and (by invitation) A C ERNSTENE and B M JACOBSON Boston, Mass

Observations by one of us (S A L) on a patient with bronchial asthma and angina pectoris in whom an attack of angina was precipitated by a therapeutic dose of epinephrine gave rise to the idea that possibly epinephrine could be employed as a diagnostic measure in patients in whom the diagnosis of angina pectoris was not certain. With this in mind, one cubic centimeter doses of a 1:1000 solution of epinephrine chloride have been given subcutaneously to eleven patients with angina pectoris and in all but one a typical anginal attack has been produced. The single exception cannot, for various reasons, be accepted as a complete failure. In some instances the pain was allowed to subside spontaneously, while in others it was brought to an end by nitroglycerine or amyl nitrite. Ten patients of the same age group as those with angina pectoris but without evidence of cardiac disease and ten normal young adults have been tested similarly, and in no instance has anginal pain been produced.

Frequent blood pressure determinations and electrocardiograms were taken in each experiment. It was originally hoped that distinctive changes might be found in the electrocardiograms of anginal patients as a result of the epinephrine, which might further serve as a diagnostic aid. In only one case, however, did any important changes develop although extrasystoles were frequently noted. There was nevertheless a striking difference in the response of the T wave in the three

groups In the patients with angina pectoris, the T-wave invariably increased in amplitude, while in the group of older controls there was, as a rule, very little change in amplitude, and in the young controls there was usually a decided diminution in height of the T-wave This work indicates that adrenalin may serve as a diagnostic test for angina pectoris Its use, however, would need to be applied with caution and discretion

*Emphysema Simulating Cardiac Decompensation* By W B KOUNTZ (by invitation) and H L ALEXANDER, St Louis, Mo

Sixty-three cases of advanced emphysema were studied In all, there were dyspnea on exertion, and some cyanosis, and in forty, dependent edema Direct examination of the cardiovascular system showed no abnormality excepting consistently increased venous pressure

Autopsies were performed in nine cases Eight of these had had dependent edema No cardiac lesions appeared The right ventricular wall was not increased either in thickness or in weight

Dyspnea in these cases is attributed to the consistently lowered vital capacity, cyanosis, to deficient pulmonary ventilation The dependent edema is presumed to be caused mainly by increased venous pressure

The rise in venous pressure in these cases was studied experimentally, and found to be due to two factors, increased intrathoracic pressure and anoxemia

An extensive emphysema in dogs was produced by inserting an adjustable ball-valve in the trachea During the development of the emphysema, periodic intrathoracic and peripheral venous pressures were taken simultaneously It was found that the venous pressure rises proportionately to the increase in intrathoracic pressure Dogs were rendered partially anoxic and a rise in venous pressure in proportion to the degree of the oxygen unsaturation of the arterial blood was observed

These observations tend to refute persistent statements that emphysema leads to right heart failure

*Chronic Infectious Non-Tuberculous Pulmonary Lesions of Childhood and Their Relation to Bronchiectasis* By F MAURICE MCPHEDRAN, Philadelphia, Pa

A number of these lesions have been observed in children during the past six years Some were seen shortly after an illness severe enough to initiate a lesion, many during recurring attacks of pneumonia from which a persisting infiltration results, others, without symptoms, were discovered by routine x-ray examination The last usually sooner or later suffer characteristic bronchopneumonic exacerbations, which are roentgenographically recordable, sometimes producing severe illness, sometimes accompanying apparently trivial coryza

The lesions predominate in the left lower lobe When early, whether right or left, they are densest close to the heart Synchronized diastolic roentgenograms accurately record slight lesions and eliminate misleading blurring due to systole

Early diagnosis and care is important to prevent advanced scarring and bronchiectasis

Among 106 colored children occurred five cases, among 325 Italians 19, among 341 other white children only 4. Five cases occurred in one family, three in each of two others. In two additional families each presenting two cases the fathers had suffered from clinical sinusitis for many years.

These lesions are commonly diagnosed tuberculosis. Differentiation presents few difficulties.

No characteristic or predominating organism has been found. Paranasal sinusitis is common.

*Clinical and Experimental Studies of the Effect of Ergotamine on Metabolism and the Circulation* By JOHN B. YOUNG, M.D., and (by invitation) W. H. TRIMBLE

Ergotamine tartrate in doses of 0.5 mgm. subcutaneously caused a small drop in pulse rate and a small rise in diastolic pressure in normal human subjects under basal conditions. No significant effect on metabolic rate or blood sugar was noticed over periods up to three hours. Severe muscle pains and soreness, nausea, headache and lassitude were common. Atropine in doses of 1.2 mgm. subcutaneously prevented or abolished the drop in pulse rate. Continued administration of ergotamine in doses of 1 mgm. by mouth, three times daily for a week had no significant effect on metabolic rate. Patients with thyrotoxicosis showed in general similar results but the effect on pulse rate and blood pressure was much more marked.

Doses somewhat larger (0.105 to 0.5 mgm. per animal), but smaller than those usually reported, were employed intravenously in trained nonnarcotized dogs. In these animals under basal conditions ergotamine caused an immediate and marked fall in heart rate which was prevented or abolished by atropine. No constant changes in oxygen consumption or blood sugar levels were observed over periods up to three hours. In the doses used ergotamine failed to prevent or diminish epinephrine hyperglycemia in these animals.

*Observations on Changes in Respiration and Circulation Occurring Coincidentally with Sensations of Faintness and Impending Syncope in Normal Individuals* By ISAAC STARR, JR. and (by invitation) LEON H. COLLINS, JR., Philadelphia, Pa.

While there has been much speculation concerning the physiology of syncope (Gowers, Hering, etc.), observations upon this condition have been limited to changes in pulse rate and blood pressure, chiefly after collapse (Cotton and Lewis). The opportunity to extend these observations came during experiments designed to determine the applicability of the ethyl iodide cardiac output method (Starr and Gamble) to the problem of the effect of posture on blood flow. The subjects, standing, inhaled ethyl iodide, blood being obtained from a vein on the back of

one hand immersed in water at 45°C. A graphic record of respiration was taken from the spirometer, blood pressure and pulse rates were observed at frequent intervals.

Four subjects experienced faintness and two collapsed. The records show that the sensation of faintness was always accompanied by hyperventilation but was usually without significant change of pulse rate, blood pressure or cardiac output. Symptoms of collapse were preceded by marked hyperventilation and accompanied by marked fall in pulse rate and blood pressure and apparent diminution of ventilation. Alveolar  $\text{CO}_2$  was very low in two such cases, in one of which improvement followed rebreathing. The cardiac output could not be determined at the time of collapse, it remained unchanged or fell but little during the period preceding.

Certain features of the physiological picture described low blood pressure without diminution of cardiac output have been produced in anesthetized cats (Dale and Evans) by hyperventilation by blowing off of  $\text{CO}_2$ , but no pronounced fall of heart rate occurred in these experiments. Other features, hyperventilation and slow pulse regularly occur directly after the production of cerebral anemia in animals (L. Hill, Pike, Schmidt, etc.), but a high rise of blood pressure usually occurred at this time. In an occasional experiment in this laboratory, occlusion of the cerebral arteries of an anesthetized cat caused prolonged hyperventilation and an elevation of blood pressure lasting only a few seconds followed by marked slowing of the pulse rate with fall of blood pressure below the initial level. These results seem essentially similar to the physiological picture we observed in impending syncope in normal individuals.

The fact that the hyperventilation precedes the fall of blood pressure and pulse rate in our subjects is evidence against the conception (Lewis) that the primary cause of syncope is reflex vagus stimulation. Our observations are consistent with the conception that syncope is caused by cerebral anemia secondary to failure of the peripheral circulation to adjust itself to the upright position and so maintain the blood supply to the brain.

#### *Skin Reactions of Patients and Normal Individuals to Protein Extracts of Streptococci*

By CLIFFORD L. DERICK and (by invitation) MARSHALL N. FULTON, Boston, Mass.

Skin tests were carried out on 366 general hospital patients, 67 infants and children in a general medical ward and 51 patients with rheumatic fever or chorea in whom the disease was either active or early in the convalescent stage. A group of apparently normal adults were similarly tested for comparison.

The purpose was to determine what proportion of these individuals would show skin sensitiveness of the tuberculin type to protein extracts of different varieties of streptococci. Test materials used were pure nitrogen-constant extracts from each of three types of streptococci—hemolytic, green-producing, and indifferent. A similarly prepared extract from Baker's yeast was used for control purposes. Varying amounts in normal saline solution were injected into the skin of the forearm. All readings were made at 24-hour intervals for 2 or more days.

The analyzed results reveal that patients with certain diseases show a high incidence of positive reactions, that there seems to be a relationship between previous infections and skin sensitiveness, and that age, except in the very young, plays a minor if any rôle

The presence of reaction is looked upon as indicating present or previous infection with streptococci and is not characteristic of any one disease

*Rate of Removal of Bacteria from the Blood in Human Disease* By REUBEN OTTENBERG, New York, N Y

A technic has been developed by which simultaneous blood cultures are made from the two internal jugular veins. In sinus thrombosis there is usually a much larger number of colonies in one vein than in the other. In 20 of the more recent cases a simultaneous blood culture was also made from an arm vein. I wish at present to point out the very small number of colonies usually found in the arm vein as compared with that jugular vein which is draining the lesion most directly.

Among 17 cases of otitic infections suspected to be sinus thrombosis there were 7 in which the colonies could be counted. In every one of these the number of colonies in the arm blood was much smaller than in one or both jugulars. Thus

*Colonies per cubic centimeter of blood*

|         | Right<br>Jugular | Left<br>Jugular | Arm vein |                        |
|---------|------------------|-----------------|----------|------------------------|
| Case 4  | 122              | 7               | 6        | Left sinus thrombosis  |
| Case 25 | 600              | 11              | 1        | Right sinus thrombosis |
| Case 32 | 250              | 240             | 1/3      | Right sinus thrombosis |

In two cases of ulcerative endocarditis the number of colonies found in the jugular veins and arm vein were approximately equal.

It is evident then that during circulation of blood through the lungs an enormous number of bacteria are removed. The number in the periphery represents only a small fraction of those which are constantly being fed into the blood stream.

*White Blood Cells in Medical Conditions* By PAUL REZNIKOFF, New York, N Y

The object of this study was to determine variations of the white blood cells and the intensity and course of "the shift to the left" (the response of young polymorphonuclear cells) during the course of illness. Total white cell and Schilling counts were performed on 177 patients on the medical wards.

These included 78 patients suffering from pneumonia and 23 having some form of upper respiratory infection. Four tabulations were made: (a) total white cell counts, (b) percentage of polymorphonuclears, (c) percentage of all cells which are young polymorphonuclears, (d) percentage of all polymorphonuclears which are young.

It was found that all patients suffering from acute infections have a shift to the

left regardless of their total white blood, total polymorphonuclear count or temperature. The increase in the relative and absolute number of young polymorphonuclears varies roughly with the intensity of the infection. The return of the shift to the right or failure to do so is the most accurate gauge of prognosis that can be obtained from blood studies.

*Arthritic Pain in Relation to the Weather* By L. G. ROWNTREE and (by invitation) E. B. RENTSCHLER and F. R. VAN ZANT, Rochester, Minn.

For a period of a year we have studied the pain in a series of arthritic patients, 377 in all. We have found a striking relationship between the pain and the presence of storms about 90 per cent of the time. Between barometric pressure and pain we have found a relationship 93 per cent of the time. In 72 per cent there has been a direct relationship, and in 21 per cent a negative, or inverse relationship. Sunshine seems to be another factor of considerable interest. We have also studied a group of arthritic "weather prophets" and found that many of them can predict weather changes 12 to 24 hours in advance. The literature has been covered in connection with this subject, but little of an exact nature has been written to date. The possible relationship of the sympathetic nervous system to this phenomenon has been introduced through the discussion of a patient in whom removal of the second, third and fourth lumbar sympathetics was done. This patient was suffering from arthritis and had always noticed a relationship between storms and increase of her pain. After the operation there was no such relationship in the lower extremities, while the upper extremities, which were also afflicted with arthritis, continued to be affected by storms.

*The Respiratory Quotient of the Obese During Reduction* By H. B. MCCLUGGAGE, and J. M. STRANG, (by invitation) and FRANK A. EVANS, Pittsburgh, Pa.

Observations made on ten obese patients undergoing reduction by dieting during an average period of fourteen weeks showed a basal R Q of less than 0.707 in 35 per cent of 112 determinations. The average R Q before dieting was 0.776 for 8 subjects, the average R Q during the dieting period was 0.728. Very low respiratory quotients appeared usually after two weeks of dieting. Only one patient failed to go below 0.707 at some time. The average respiratory quotient reaches its lowest level during the third month when 19 determinations on 7 patients averaged 0.701.

The lowest observed R Q 0.632, was obtained at the eighth test on a patient on whom 6 of 13 tests ran low. Another patient, on whom 13 of 26 determinations were low, had values of 0.653, 0.650 and 0.657 on three successive weeks and a minimum of 0.640. The average of the lowest respiratory quotients observed on 9 patients was 0.661.

These very low respiratory quotients do not correspond to (1) the periods of most rapid weight loss, or (2) the presence or absence of acetone bodies in the urine. A large number of blood CO<sub>2</sub> combining power determinations have fallen within normal limits.

*Observations Concerning Intracranial Circulation in the Human Subject*

WILLIAM G. LENOX, Boston, Mass.

The oxygen and carbon dioxide content of arterial blood and of venous blood from the internal and external jugular and the basilar veins was measured in a number of patients. In these subjects, the gaseous content of blood from the external jugular vein approached that of arterial blood. In many of the individuals there was a wide discrepancy between the gaseous content of blood from the internal jugular and the basilar veins. The average measurements for the group, however, were about the same.

In experiments conducted with Dr. Soma Weiss, it was observed that the intravenous injection of histamine caused the venous blood from each of these veins to become more arterial like. Apparently cerebral vessels respond to stimulation in much the same way as vessels of the skin.

*The Cerebral Circulation. V. The Action of Histamine* By H. S. FORBES

H. G. WOLFF (by invitation) and STANLEY COBB, Boston, Mass.

By the method developed by one of us for observing the pial vessels through a window in the skull, studies have been made of the effect of histamine on cerebral circulation. It was observed that the vessels of the brain react to a venous injection of histamine in a very different manner, depending on whether the animal is under ether or amytal anesthesia. Under ether the vessels of the brain are already dilated and so do not dilate to any extent, if at all, following intravenous injection of histamine. On the contrary they often become narrowed and the cerebrospinal fluid pressure falls.

Local application of histamine to the surface of the brain always results in dilatation of the pial vessels, without noticeably affecting intracranial or systemic vascular pressures. Intravenous injection of histamine (in animals under amytal) causes great pial artery dilatation in spite of a coincident fall in systemic arterial pressure.

*Studies in the Etiology and Iodine Reactions of Simple Goiter in Rabbits*

BRUCE WEBSTER (by invitation) and ALAN M. CHESNEY, Baltimore, Md.

Further studies have been carried out to determine the etiology of an epidemic of simple goiter in rabbits. These suggest that the basic factor in this instance is a dietary one. The addition of 7.5 mgm. of iodine per week to the goiter-producing diet has been found sufficient to prevent the development of goiter. Improving the sanitary conditions under which the animals were kept, by altering the manner of caging so as to reduce the fecal contamination of the food, was found to exert only slight protective action against the goiter-producing influence.

Previous studies have shown that the administration of iodine to goiter-bearing rabbits is followed by a marked increase in heat production. Studies have been made in an effort to quantitate the production of thyroid hormone against available iodine. Raises in heat production were studied after the administration

left regardless of their total white blood, total polymorphonuclear count or temperature. The increase in the relative and absolute number of young polymorphonuclears varies roughly with the intensity of the infection. The return of the shift to the right or failure to do so is the most accurate gauge of prognosis that can be obtained from blood studies.

*Arthritic Pain in Relation to the Weather* By L. G. ROWNTREE and (by invitation)

E. B. RENTSCHLER and F. R. VAN ZANT, Rochester, Minn.

For a period of a year we have studied the pain in a series of arthritic patients, 377 in all. We have found a striking relationship between the pain and the presence of storms about 90 per cent of the time. Between barometric pressure and pain we have found a relationship 93 per cent of the time. In 72 per cent there has been a direct relationship, and in 21 per cent a negative, or inverse relationship. Sunshine seems to be another factor of considerable interest. We have also studied a group of arthritic "weather prophets" and found that many of them can predict weather changes 12 to 24 hours in advance. The literature has been covered in connection with this subject, but little of an exact nature has been written to date. The possible relationship of the sympathetic nervous system to this phenomenon has been introduced through the discussion of a patient in whom removal of the second, third and fourth lumbar sympathetics was done. This patient was suffering from arthritis and had always noticed a relationship between storms and increase of her pain. After the operation there was no such relationship in the lower extremities, while the upper extremities, which were also afflicted with arthritis, continued to be affected by storms.

*The Respiratory Quotient of the Obese During Reduction* By H. B. McCLUGGAGE, and J. M. STRANG, (by invitation) and FRANK A. EVANS, Pittsburgh, Pa.

Observations made on ten obese patients undergoing reduction by dieting during an average period of fourteen weeks showed a basal R. Q. of less than 0.707 in 35 per cent of 112 determinations. The average R. Q. before dieting was 0.776 for 8 subjects, the average R. Q. during the dieting period was 0.728. Very low respiratory quotients appeared usually after two weeks of dieting. Only one patient failed to go below 0.707 at some time. The average respiratory quotient reaches its lowest level during the third month when 19 determinations on 7 patients averaged 0.701.

The lowest observed R. Q. 0.632, was obtained at the eighth test on a patient on whom 6 of 13 tests ran low. Another patient, on whom 13 of 26 determinations were low, had values of 0.653, 0.650 and 0.657 on three successive weeks and a minimum of 0.640. The average of the lowest respiratory quotients observed on 9 patients was 0.661.

These very low respiratory quotients do not correspond to (1) the periods of most rapid weight loss, or (2) the presence or absence of acetone bodies in the urine. A large number of blood  $\text{CO}_2$  combining power determinations have fallen within normal limits.

or paroxysmal than that due to other causes. One fourth of the cases in this series were of this type.

The cardiac manifestations of Graves' disease may dominate the picture and obscure the underlying thyroid disorder, they may even antedate all other recognizable signs of the Graves' disease causing them.

Digitalis is less effective than in fibrillation not due to Graves' disease. The amounts tolerated are larger, and the beneficial effects are less striking.

Following successful treatment of the underlying Graves' disease, recovery from fibrillation is usually prompt and striking. Restitution of the heart to normal is often complete. Quinidine is often very useful in restoring permanent normal rhythm.

Cardiac hypertrophy is seldom pronounced. Enlargement, when present, is due chiefly to dilatation.

In some respects the cardiac disturbances of Graves' disease resemble those of toxemia of acute infection.

Although it is difficult to estimate the relative importance of mechanical and toxic factors, the infrequency of hypertrophy and the character of the disturbances suggest that the latter is of major importance.

*A Comparison of the Coronary and Digitalis T Waves. An Electrocardiographic Study.* By WALTER W. HAMBURGER, Chicago, Ill.

Inversion of the T wave of the human electrocardiogram, is one of the most frequent findings in clinical electrocardiography. The two most frequent causes of T wave inversion are coronary disease and digitalization. The character of inversion is different in these two conditions: coronary inversion shows the typical cove shape (Pardee); digitalis inversion appears as an immediate straight line depression of the R-T interval. A coronary-digitalis wave appears as a merging of the two. Examples of these three types of inversion are presented. The clinical importance of recognition of these various types is emphasized.

*Skin Tests with Pneumococcus and B. influenzae Antigens in Influenza and Pneumonia.* By ALLAN K. POOLE, and JOHN H. BUMSTEAD, (by invitation) and FRANCIS G. BLAKE, New Haven, Conn.

The study was undertaken with the purpose of determining the skin reactivity or allergy of patients with uncomplicated influenza and with pneumonia to antigens prepared from *Pneumococcus* Type I, *Pneumococcus* Type II and *B. influenzae*. Patients with influenza (23 cases) consistently showed marked reactivity to all three antigens in the acute febrile stage; lessened reactivity during the first week of convalescence and in the majority no reactivity after the 20th day. Patients with pneumonia (32 cases) showed a striking contrast. With one exception, a case of streptococcus pneumonia, no case reacted to the pneumococcus antigens during the acute stage, but those retested became reactive during convalescence. Forty per cent reacted to *B. influenzae* antigen during the acute

single small doses of potassium iodide to animals in which the degree of hyperplasia of the thyroid gland had been previously determined by biopsy. The increase in heat production appeared to vary directly with the amount of iodine exhibited, as did also the extent of involution of the gland. This relationship apparently held true until involution was complete.

*The Effect of Liver and of Liver Extract upon Appetite* By A. C. CURTIS (by invitation) and L. H. NEWBURGH, Ann Arbor, Mich.

The addition of 2 per cent protein as dried ether extracted liver to a basic rat diet containing 2 per cent vitamin B powder (Harris) causes greater energy ingestion and better growth than does the addition to this diet of 2 per cent protein in the form of casein.

If vitamin B powder is omitted from both diets, greater loss of appetite and weight occurs in the group of rats eating the basic diet containing 2 per cent protein in the form of casein. All animals of both groups develop polyneuritis.

The addition of 2 per cent liver extract (Lilly) to the basic rat diet plus one yeast vitamin powder tablet daily (Harris) causes much greater ingestion of energy and much better growth than was observed when 2 per cent dried ether extracted liver protein or casein protein was added to the basic rat diet.

When the basic diet, containing added liver extract (Lilly), ether extracted liver protein, or casein protein, is supplemented by sufficient yeast vitamin powder (Harris), energy ingestion and growth are equal.

If basic rat diets, containing autoclaved ether extracted liver and liver extract, are fed without yeast vitamin powder, polyneuritis develops in all experimental animals.

When 2 per cent liver extract (Lilly) is added to the basic diet, which contains no other source of vitamin B, growth and energy ingestion are approximately normal. The replacement of liver extract by equal amounts of yeast vitamin powder (Harris) causes less growth and less energy ingestion.

A patient with pernicious anemia was fed 24 grams of autoclaved liver extract (Lilly) daily for 6 days and did not experience a rise in reticulated cells or red cells. At the end of this period 24 grams of unaltered liver extract (Lilly) was fed daily with a reticulated cell rise and red cell rise occurring in the usual time.

1. The factor in liver that effects appetite is, seemingly, that fraction of vitamin B.

2. Liver extract (Lilly) appears to contain the appetite factor of vitamin B in concentrated form.

*Auricular Fibrillation in Graves' Disease* By PAUL S. BARKER, and ANN BOHNING (by invitation) and FRANK N. WILSON, Ann Arbor, Mich.

A study of 107 unselected cases of auricular fibrillation due to Graves' disease, and observed from 1923 to 1927, leads to the following conclusions:

The auricular fibrillation due to Graves' disease is more likely to be transient.

higher percentage than normal of abnormal sized red blood cells in the peripheral blood of patients having carcinoma of the breast or prostate, and with a normal blood count suggests the possibility of bone marrow metastasis. Anemia from other causes must be eliminated when the red blood cell count is lower than normal before the abnormal sized cells can be considered as suggestive of bone metastasis, although cells larger than normal are of greater significance.

*A Comparison of Volume Index and Diameter of the Red Cells in Different Types of Anemia* By RUSSELL L. HADEN, Kansas City, Kans.

Accurate knowledge of the size of the red cells is of fundamental importance in the study of the anemias. The size of the average cell may be determined from the volume index which gives the volume of the average cell. The size may also be determined by measuring the diameter of a representative group of cells. Fifty cases each of pernicious anemia and of secondary anemia have been studied by these two methods. The data obtained and the advantages and disadvantages of the two methods are discussed.

*The Measurement of Temperatures in the Bladder, Cervix and Rectum of Patients Receiving Diathermy Treatment of the Pelvis* By F. W. BISHOP and E. K. RITCHIE, (by invitation) and S. L. WARREN, Rochester, N. Y.

The pelvis was treated by passing a high frequency or diathermy current between a speculum in the vagina and a sacral plate.

Temperatures were measured by thermocouples placed in the cervix, bladder, rectum and elsewhere.

When the high frequency is turned on, all of the regions measured showed a simultaneous rise in temperature to a plateau which was maintained while the current was applied. On turning off the current there was a similar drop in the temperatures to the original value. Each state of equilibrium was reached in about five minutes.

With currents of from 1800 to 2400 milliamperes in average patients, local temperatures as high as 41°C. were measured and maintained at this level during treatment. The temperatures in the rectum, cervix and bladder were very nearly the same. Individual patients varied considerably in the amount of increase in temperature found, the average attained being around 42°C. Body temperature (mouth) was elevated only 0.5° to 1.0°C. Blood pressure was unaffected.

*The Question of Arsphenamine Resistant Syphilis* By JOSEPH EARLE MOORE, and (by invitation) HARRY M. ROBINSON, Baltimore, Md.

A survey of the literature indicates that in Germany and perhaps elsewhere in Europe, early syphilis reacts to arsphenamine treatment less favorably now than in the years before the War, in the sense that secondary relapses are more frequent, the blood Wassermann is not so rapidly reduced to negative, and an increasing number of patients whose lesions fail to heal under arsphenamine treatment are

stage Those retested in convalescence retained or developed reactivity In brief, patients with influenza appear to show heightened reactivity to bacterial antigens during the acute stage and tend to lose this after recovery, patients with pneumonia show little reactivity during the acute stage and tend to become more reactive after recovery The interpretation awaits further studies now being carried on

*Venous Pressure in General Anaesthesia* By O O MEYER (by invitation) and WM S MIDDLETON, Madison, Wis

Forty-two individuals under general anaesthesia have been studied with a view to determining the influence of such agents The results may be summarized as follows

- 1 Coincident with the increased muscular effort and altered respiratory function of the induction period, pronounced increases in venous pressure are noted

- 2 Thereafter a subsidence occurs to a plateau of venous pressure somewhat elevated above the normal, which is maintained throughout the maintenance of anaesthesia

- 3 Alterations in this plateau apparently depend upon reflex stimuli, usually in a period of lightened anaesthesia

- 4 Elevations in venous pressure occur on release from anaesthesia dependent upon such acts as retching, vomiting, etc

- 5 Carbon dioxide inhalations operate apparently independently to increase venous pressure

These results are not interpreted as bearing a causal relation to the circulatory accidents of general anaesthesia and surgical operation Indeed, the demands of ordinary life with its occasional calls for sharp elevation in venous pressure, as in the act of defecation, may lead to much greater strain on the right heart than has been noted in these venous pressure determinations under properly administered general anaesthetics

*Red Blood Cell Measurements in the Diagnosis of Metastatic Cancer in the Bone Marrow* By RAPHAEL ISAACS and (by invitation) MADELAINE R BROWN, Ann Arbor, Mich

In patients with carcinoma with metastasis to the bone marrow, red blood cells smaller or larger than normal appear in greater frequency in the blood stream than in normal persons Seven out of thirteen patients showed a disproportionate percentage of cells larger than normal and all of them showed a relative increase in the percentage of cells smaller than normal at some stage in their disease In no case did the degree of microcytosis approach that of the blood after hemorrhage or in secondary anemia from other cause With the present data no evident correlation between the degree of variation in the size of the cells and the extent of the metastasis, can be made, due, probably, to the difficulty in judging the true degree of the marrow involvement The presence of a relatively

higher percentage than normal of abnormal sized red blood cells in the peripheral blood of patients having carcinoma of the breast or prostate, and with a normal blood count suggests the possibility of bone marrow metastasis. Anemia from other causes must be eliminated when the red blood cell count is lower than normal before the abnormal sized cells can be considered as suggestive of bone metastasis although cells larger than normal are of greater significance.

*A Comparison of Volume Index and Diameter of the Red Cells in Different Types of Anemia* By RUSSELL L. HADEN, Kansas City, Kans

Accurate knowledge of the size of the red cells is of fundamental importance in the study of the anemias. The size of the average cell may be determined from the volume index which gives the volume of the average cell. The size may also be determined by measuring the diameter of a representative group of cells. Fifty cases each of pernicious anemia and of secondary anemia have been studied by these two methods. The data obtained and the advantages and disadvantages of the two methods are discussed.

*The Measurement of Temperatures in the Bladder, Cervix and Rectum of Patients Receiving Diathermy Treatment of the Pelvis* By F. W. BISHOP and E. K. RITCHIE, (by invitation) and S. L. WARREN, Rochester, N. Y.

The pelvis was treated by passing a high frequency or diathermy current between a speculum in the vagina and a sacral plate.

Temperatures were measured by thermocouples placed in the cervix, bladder, rectum and elsewhere.

When the high frequency is turned on, all of the regions measured showed a simultaneous rise in temperature to a plateau which was maintained while the current was applied. On turning off the current there was a similar drop in the temperatures to the original value. Each state of equilibrium was reached in about five minutes.

With currents of from 1800 to 2400 milliamperes in average patients, local temperatures as high as 44°C were measured and maintained at this level during treatment. The temperatures in the rectum, cervix and bladder were very nearly the same. Individual patients varied considerably in the amount of increase in temperature found, the average attained being around 42°C. Body temperature (mouth) was elevated only 0.5° to 1.0°C. Blood pressure was unaffected.

*The Question of Arsphenamine Resistant Syphilis* By JOSEPH and (by invitation) HARRY M. ROBINSON, Baltimore, Md.

A survey of the literature indicates that in Europe, early syphilis reacts to arsphenamine treatment in the years before the War, in the sense that as the blood Wassermann is not so rapidly reduced in number of patients whose lesions fail to heal.

appearing. It seems probable that this situation is due less to any decrease in the therapeutic activity of arsphenamine, or to the development of so-called arsphenamine-fast strains of spirochetes, than to the reaction of the individual patient to treatment. Composite Wassermann curves of 1116 patients with early syphilis studied from 1914 to 1927 in the Syphilis Clinic of the Johns Hopkins Hospital show that there is no significant difference in serologic results in recent as compared with earlier years. Relapses of a secondary type are, in our material, no more frequent in 1925-1927 than in 1914-1923. True arsphenamine-resistant syphilis is not increasing in this locality. Patients with lessened resistance to syphilitic infection as measured by the standards of "seronegative early secondary syphilis" or "premature Wassermann negativity" are no more frequent in our material now than in earlier years. There is no evidence to indicate that, in this part of the United States, arsphenamine is less efficacious than formerly in the treatment of syphilis, or that arsphenamine resistant syphilis is increasing. Certain general aspects of the phenomenon of arsphenamine-resistance are discussed.

*The Relation of Recovery and Development of Humoral Immunity in Pneumococcus Pneumonia in Children* By C O'DONOVAN, JR., (by invitation) and JAMES D TRASK, New Haven, Conn

In fixed type pneumococcus pneumonia in adults recovery has been shown to be associated with the development of type specific humoral antibodies.

Children are supposed to recover from this disease more regularly than adults. Accordingly a study was made of the development of type specific agglutinins in the blood serum of 19 children with the disease. There were 12 Type I, 2 Type II and 5 Type III cases, all recovered. However the development of specific antibodies was found to be significantly less marked than has been reported for adults.

*The Tissue Transfer Method of Determining Cure of Syphilis in Man* By HUGH J MORGAN and (by invitation) JAMES L ALLOWAY, Nashville, Tenn

Brown and Pearce have demonstrated the frequency with which treponemas localize in the lymph nodes of infected rabbits and remain there long after the acute stage of the infection has passed. This lymph node reservoir for treponemas is used by workers in experimental syphilis as a means for recovering organisms from animals without active lesions. This may be readily done by emulsifying a lymph node and injecting it into the testicle of a normal rabbit. An acute orchitis develops in the inoculated animal or in subsequent transfers. After treatment of experimental syphilis in the rabbit, failure to recover organisms by this method has been interpreted as an indication of cure.

In 1927 Chesney and Kemp stimulated interest in the possibility that the tissue transfer method of determining cure of experimental syphilitic infection might be applicable to syphilis in man. They applied the method to three patients and

were unable to recover treponemas from lymph nodes after suitable treatment. This work raised the very important question as to whether or not these negative results indicate biological cure.

In order to throw some light on this subject we have undertaken a study of the recoverability of treponemas from lymph nodes of untreated patients in different stages of the disease. It was felt that if, in untreated cases of syphilis, as in the experimental disease, treponemas could always be recovered from lymph nodes, then the positive or negative findings after treatment would be of great significance and might be used as criteria of cure.

In all instances enlarged lymph nodes (inguinal, epitrochlear or cervical) were removed under novocaine anesthesia, immediately cut into small bits and ground in a mortar with a small amount of warm saline solution. The resulting emulsion was injected in equal amounts into the right testicles of two normal rabbits. The animals were kept under constant observation. Dark field examinations were made with specimens from all visible or palpable scrotal or testicular lesions and with all subsequent testicular emulsions used for transfers. Animals which developed no lesions at the end of ninety days were sacrificed and the right testicle of each was removed, emulsified, examined by dark field and injected intratesticularly into two normal rabbits. No tissue transfer experiment was declared negative until transfers had thus been made through two sets of rabbits.

Our findings are summarized in the following table.

TABLE 1

*The recovery of treponemas from lymph nodes in untreated syphilis (tissue transfer method)*

| History number | Clinical diagnosis                  | Tissue transfer |          |
|----------------|-------------------------------------|-----------------|----------|
|                |                                     |                 |          |
| 11238          | Primary syphilis                    | Positive        |          |
| 11806          | Primary syphilis                    | Positive        |          |
| 11405          | Secondary syphilis (late)           |                 | Negative |
| 9655           | Latent syphilis                     |                 | Negative |
| 11569          | Latent syphilis (congenital?)       |                 | Negative |
| 11890          | Tertiary syphilis (leg ulcers)      |                 | Negative |
| 12227          | Tertiary syphilis (skin recurrence) | Positive        |          |
| 10987          | Tertiary syphilis (gumma of knee)   |                 | Negative |
| 10829          | Neurosyphilis (tabes dorsalis)      |                 | Negative |

We conclude from this study that the tissue (lymph node) transfer method of determining the presence or absence of syphilitic virus in experimental infection does not invariably give positive results when applied to patients with proven untreated syphilis. Therefore, in human syphilis the method is unreliable. In a given case negative results before any treatment is instituted is of no diagnostic significance. After treatment is instituted negative results cannot be interpreted as indicating biologic cure. It is suggested that this discrepancy in results with the tissue transfer method in human and experimental syphilis is probably due to

the fact that in the latter the virus is usually one thoroughly adapted to the rabbit by previous animal passage Dosage also is probably important and this factor is now being studied by us

*Observations on the Apparent, Adaptability of the Body to Infection, Unusual Hardships, Changing Environment and Prolonged Strenuous Exertion* By BURGESS GORDON and (by invitation) JOHN C BAKER, Philadelphia, Pa

Competitors in a transcontinental foot race (March, April and May, 1928) who walked or ran 3482 miles in 84 consecutive days were studied before, during and after the contest In histories obtained from 174 contestants a wide variation in age, physical condition, preliminary training and diet was noted Infections, injuries and experiences common in frontier life of 75 years ago occurred during the race The chief causes for withdrawal from the contest were so-called "shin splints," resulting from myositis and periostitis Various fads and fancies in diet were advocated by the runners at the beginning of the race As the contest progressed, however, meat and certain vegetables could not be supplied as desired Carbohydrate became the predominant food Several competitors consumed no meat during the entire race and others none after the first 500 miles It was noted that carbohydrate in the form of oranges, dates, figs, honey, dextrose and cake between meals was taken by most runners after the first few hundred miles, apparently in order to relieve hunger and fatigue

Observations made after the race, which included microscopic and chemical studies of the blood and urine, roentgenographic, electrocardiographic, ophthalmoscopic, orthopedic and laryngeal examinations were essentially negative as would be expected from a group of apparently normal individuals who had followed less strenuous forms of exercise Correspondence during the past eight months shows that 84 contestants have suffered no apparent untoward effects

In considering the condition of the runners at different periods during the race it would appear that it is extremely difficult to estimate the potential endurance of an individual or to predict the degree of resistance to infection

*A Clinical Metabolic Study of "Obstinate" Cases of Obesity* By SOLOMON STROUSE and (by invitation) C C WANG, Chicago, Ill

This work was undertaken on patients who had resisted supposed strict dietary procedures at home They were then established in the metabolic clinic at the hospital and placed under complete control A noticeable contrast was shown and the patients could be made to lose weight mathematically according to schedule

*The Clinical Interpretation of Diabetes* By JOHN R WILLIAMS, Rochester, N Y

The following is an abstract of a study of upwards of five hundred diabetics with reference to a better means of control and interpretation It is commonly believed that insulin causes the burning or utilization of 1.5 to 2 grams of glucose

per unit For the past six years the writer has coöperated with the Eli Lilly Company in the clinical testing of insulin In this work one patient, whose metabolism is fairly constant, and who is highly trained has been used as a test case The results obtained have been carefully checked and have invariably corresponded closely with the assays of the Lilly Company The work which will be reported in a later communication indicates that one unit of insulin causes the utilization of approximately 4 grams of glucose rather than 2 grams as is commonly assumed

The diet of normal individuals contains from 300 to 800 grams of glucose forming food One unit of insulin will burn approximately 4 grams of glucose The normal pancreas makes from 75 to 200 or more units of insulin daily according to the food intake of the individual When the pancreas fails to make sufficient insulin to care for the glucose content of the diet diabetes results That portion of the glucose which is not utilized and which exceeds the storage capacity of the body will be eliminated as urine sugar The difference between the amount of glucose ingested and that excreted in the urine is the glucose utilization of the patient The glucose utilization factor in a well treated case of diabetes is fairly constant and may be increased by the administration of insulin In either a normal individual or a diabetic the number of grams of glucose utilized, divided by four, will give the number of units of insulin made by the individual This figure hereafter designated as the insulin coefficient is a fairly constant factor in the diabetic Example—The diet contains 100 grams of glucose, 20 grams are eliminated in the urine, therefore 80 grams are utilized The pancreas makes 20 units of insulin

To determine the coefficient of a patient taking insulin, the number of units administered must be subtracted from the total number of units required for the utilization of the food Thus in the above case if the patient were receiving 8 units daily his coefficient would be 20 minus 8 or 12 Written IC 12 The insulin coefficient is a fairly constant factor and changes slowly with improvement or decline It is not materially affected by occasional violations in diet even in the presence of large amounts of urine sugar and high blood sugar levels, nor is it influenced by insulin administration

A severe diabetic is one whose insulin coefficient ranges below 20, moderately severe cases range from 20 to 35 mild cases from 35 to 75 A diabetic may have a minus coefficient Example The diet contains 100 grams of glucose, the urine 20 grams of sugar 30 units of insulin are being administered daily Insulin coefficient is -10 There are several explanations for this minus coefficient (a) The insulin may be administered in too large a dose, in excess of the body's requirements at a given time and is eliminated in the urine, without serving any useful purpose, or (b) an infection or some other unsuspected disease may be present which destroys insulin, or (c) there may be an error in insulin administration or (d) in diet The insulin coefficient is a more valuable guide to the progress of an infection in diabetes than is the temperature curve or the leucocyte count It has

great prognostic value. It affords a most reliable check on the integrity of the patient who violates his diet or insulin dosage. It is by far the best method of measuring the progress of a case and the efficiency of the treatment. A patient who can raise his coefficient from 5 to 10 units in a year is making satisfactory progress.

In making tests to determine the insulin coefficient, the patient should be thoroughly instructed as to its significance and the necessity for exact cooperation. The diet should be constant for at least three days if possible. The urine should be saved and measured with care. Urine sugars should be accurately determined. A slowly rising midmorning blood sugar indicates that the patient is gradually exceeding his capacity to utilize glucose. The insulin coefficient is a far better method of differentiating spurious diabetes and renal diabetes from true diabetes than is the so-called glucose tolerance test. A glucose tolerance test is about as useful and reliable a method of determining the efficiency of the pancreas as is the "hundred yard dash in eleven seconds," a measure of the physical ability of an individual to walk five miles at a leisurely gait.

At the present time there is no clinical method in use which enables clinicians in one community to compare the efficiency of their methods with the clinicians of another community. Likewise there is no clinical method in use which enables physicians to accurately contrast or evaluate different therapeutic measures. The insulin coefficient method meets these requirements.

*Paroxysmal Bundle Branch Block* By BENJAMIN M. BAKER (by invitation) and EDWARD P. CARTER, Baltimore, Md.

An example of paroxysmal bundle branch block will be presented. The patient, when first seen, showed paroxysmal auricular fibrillation and paroxysmal bundle branch block. It was noted that during the periods of delay in intraventricular conduction the cardiac rate was above 85 to the minute. When the rate fell below this figure normal sequential rhythm with normal intraventricular conduction occurred.

Under rest and the administration of digitalis normal sequential rhythm with normal intraventricular conduction was established which constantly altered to bundle branch block when the cardiac rate was appropriately increased by various measures, thus indicating that, at least, one control of the spread of the excitation wave was the auricular rate. Delay in intraventricular conduction occurred both with and without fibrillation of the auricles.

Several galvanometric records will be shown portraying in a single lead transition from normal sequential rhythm with normal intraventricular conduction to bundle branch block.

Studies are now in progress to determine the part played by anoxemia in intraventricular conduction.

# THE INFLUENCE OF THE SYMPATHETIC NERVOUS SYSTEM ON THE CAPILLARIES DURING PASSIVE CONGESTION

By J HAMILTON CRAWFORD

*(From the Departments of Physiology and Medicine, Long Island College Hospital)*

*(Received for publication April 8 1929)*

With the discovery of vasomotor nerves by Claude Bernard the importance of the autonomic nervous system in circulatory regulation came to be recognized. The action of the sympathetic nerves on the arteries and arterioles was definitely established but it has taken many years for any action by them on the capillaries to become accepted. Indeed the capillaries were considered merely passive endothelial tubes whose variation depended on changes which took place in the other parts of the vascular system. In 1860 Beale (1) described nerves accompanying the capillaries and Glaser (2) considered these to be sympathetic nerves. The connection of these nerves to the vessel wall, however, still remains uncertain. Among the early papers Steinach and Kahn (3) presented the most positive evidence that nerve stimulation could influence the capillaries independent of its action on other vessels. They showed that stimulation of the third, fourth and fifth thoracic nerve roots at their exit from the cord produced emptying of the capillaries in the nictating membrane of the frog when the circulation was at a standstill. Krogh, Harrop and Rehberg (4) confirmed this by stimulating the lower ganglia (8-10) of the sympathetic in the frog while studying the vessels in the web under the microscope. They found that the arterioles contracted first and a few seconds later the capillaries contracted independently. The first evidence of capillary contraction following nerve stimulation in mammals was presented by Hooker (5), who stimulated the cervical sympathetic in the cat while he watched the capillaries in the depilated ear under the microscope. He observed a contraction of the capillaries independent of that of the arterioles. The most conclusive evidence of the independent contractility of the

capillaries following nerve stimulation is that brought forward recently by Harris and Marvin (6). They studied the effect of stimulation of the cervical sympathetic on the vessels in the ear of the albino rabbit. They were able to confirm Hooker's observations but they also showed that stimulation of the sympathetic caused emptying of the capillaries when the circulation was brought to a standstill by obstructing the arterial supply. Under the conditions of their experiment the effect of contraction of the arterioles or venules on the size of the capillaries would obviously have been to distend them, and not to cause them to disappear. As the result of the investigations described there can be little doubt that the sympathetic nervous system can cause contraction of the capillaries independent of its action on the other parts of the vascular system.

The evidence for a supply of vasodilator nerves to the capillaries is much less certain. Doi (7), Krogh, Harrop and Rehberg (4) and Langley (8) (9) have studied this problem and believe that such exist. They all used frogs, except Langley who used the cat's paw, and found that peripheral stimulation of posterior root ganglia or sensory nerves produced vasodilation. They endeavored to rule out the action on the arterioles by various means, Doi by dilating the arterioles with acetylcholine and Langley by occluding the circulation before applying the stimulus. Lewis and Marvin (10) repeated Langley's experiment in cats in which the sympathetic nerves had degenerated and still obtained dilatation.

Knowing the importance of vasomotor reactions to the efficient working of the normal body and having established the powerful influence of the autonomic nervous system on these reactions in all parts of the vascular system, it is important to find out whether variations in them occur in certain pathological conditions in which the flow in the vessels is abnormal. A fruitful field of investigation seemed to be the study of the vascular reactions of the capillaries during passive congestion. The abnormality of the blood flow in the capillaries in cases of advanced heart disease with chronic passive congestion and cyanosis has been described frequently. It has seemed to the author that these patients as compared to normal individuals showed a delayed and even diminished response to various stimuli such as the sudden change from a hot to a cold temperature or vice versa, fright,

etc. If this is so its importance to the organism must be very great. In order to see whether this was so the effect of stimulation of the cervical sympathetic on the capillaries of the ear during passive congestion has been compared to the normal reaction.

Albino rabbits of 1.5 to 2 kilos were used for the experiments. Ether was used as the anesthetic. At first considerable difficulty was experienced in obtaining a satisfactory production of congestion. Compression of the veins in the neck proved unsatisfactory as the collateral circulation was too free. Compression of the base of the ear would have sufficed but by this means a reduction of the arterial supply would have been obtained also. Finally the problem was solved by dissecting out the main ear veins and producing the requisite amount of traction on them by means of loops of thread passed under the veins. The sympathetic nerve was exposed on the side corresponding to the ear used and was stimulated by faradic current. The ear was fixed flat on the stage of a Leitz microscope and mineral oil was applied to its surface. The magnification used to study the capillaries was 40 times. Light was provided by a 5 ampere direct current arc lamp. It passed through a condenser and then a filter which contained a solution composed of a mixture of copper sulphate and potassium dichromate. This solution was green in color so that besides removing the heat rays it provided a better contrast between the capillaries and the tissues than was given when ordinary light was used. The light was reflected from the mirror of the microscope through the rabbit's ear. By means of this a very satisfactory view of the small vessels in the ear was obtained.

When the preparation had been set up the capillaries were watched under the microscope while an assistant stimulated the sympathetic. The efficacy of the latter was controlled by observation of the pupil. The time during which stimulation was applied was measured by a stop-watch. At first the changes were studied under normal conditions, then congestion was produced for a period varying from fifteen to twenty minutes and the sympathetic again stimulated. Finally observations were repeated from time to time after the venous obstruction had been removed. As far as possible the same capillaries were observed during each period.

Four experiments of this nature were performed and all gave the

same result. In the normal state the effect was to produce after a short latent period a marked pallor due to contraction of the arterioles. When this occurred the corpuscles in the capillaries became clumped together and the flow stopped. After a short but definite interval the corpuscles again began to move toward the vein and were finally expelled so that the capillary disappeared from view. During this latter period no fresh corpuscles were seen to enter the capillary from the arteriole. There was no doubt that the two changes described were separate processes. The latent period after which these changes took place was practically constant in repeated observations. When venous congestion was produced the ear became definitely cyanotic. The capillaries were dilated and their flow markedly altered. All gradations were seen between complete stasis and those in which there was little change from normal. The rate of flow was much reduced in the majority and was comparable to the condition which had been observed in advanced cases of heart disease (11) (12).

The flow in the different capillaries varied from time to time as had been seen also in heart cases. The capillaries particularly studied were those in which the rate of flow was considerably reduced. In these no contraction was obtained from stimulation even when it was continued for five or six times as long as had been necessary to cause disappearance of the capillaries under normal conditions. Some capillaries were observed in which the flow was only moderately reduced and in these contraction took place after a prolonged latent period. In those in which the flow was almost normal the latent period was only slightly increased. On removing the venous obstruction it was found that gradual recovery took place. Capillaries which had failed to contract during the period of obstruction began to contract after a long latent period. The latent period became less and less until finally it reached the normal level.

The following is the protocol of a typical experiment.

Albino rabbit. Weight 1.5 kilos. Ether anesthesia. Main ear veins in right ear dissected out and loose threads passed round them. Right cervical sympathetic exposed in the neck for stimulation. Right ear placed on the stage of the microscope and examined.

| Time  | Duration of stimulation           | Rate of blood flow                            | Result         |
|-------|-----------------------------------|---|----------------|
|       | <i>seconds</i>                    |   |                |
| 10 31 | 7                                 | Normal  | Contraction    |
| 10 33 | 8                                 | Normal  | Contraction    |
| 10 35 | 7                                 | Normal  | Contraction    |
|       | Venous obstruction produced 10 37 |   |                |
| 10 40 | 13                                | Capillary observed showed only slight slowing | Contraction    |
| 10 42 | 24                                | Marked slowing                                | No contraction |
| 10 46 | 32                                | Marked slowing                                | No contraction |
| 10 51 | 28                                | Marked slowing                                | No contraction |
| 10 57 | 15                                | One vessel with slight slowing                | Contraction    |
|       |                                   | Those with marked slowing                     | No contraction |
|       | Venous obstruction removed 10 58  |   |                |
| 11 01 | 15                                | Slight slowing                                | Contraction    |
| 11 06 | 10                                | Almost normal                                 | Contraction    |
| 11 12 | 6                                 | Normal  | Contraction    |
| 11 33 | 8                                 | Normal  | Contraction    |
| 11 36 | 7                                 | Normal  | Contraction    |

There was little doubt that the changes which took place in the capillaries were independent of those taking place in the arterioles. However, it was felt that if a substance could be used which dilated the arterioles but left the capillaries unaffected this possibility could be definitely ruled out. For this purpose acetylcholine was used. Taveau and Hunt (13) first showed the powerful vasodilator action of this substance in extremely dilute solution and Hunt (14) later proved that the action was independent of vasomotor nerves. Dale and Richards, (15) in their classical paper in which they analyzed the action of various vasodilator substances, found that this drug acted powerfully on the arterioles but they were unable to find any evidence of an action on the capillaries. They also showed that the changes produced were not dependent on vasomotor nerves. Acetylcholine has the further advantage that it produces a very marked vasodilatation of the cutaneous vessels. The effect of a single injection of acetylcholine passes off very rapidly so that it was necessary to give a continuous injection in order to maintain a low blood pressure during the entire period of stimulation. A solution of acetylcholine

(1 cc = 0.002 mgm) was allowed to flow from a burette into the jugular vein at a rate which it had been found in experiments on other rabbits of similar weight would maintain the blood pressure at 20–30 mm Hg below normal. It would have been preferable to have had the blood pressure reading on the animal used for the experiment but the many other details which required attention and the fact that the head was used for observation rendered it advisable to eliminate this.

Two experiments were performed in which the procedure was the same as in the previous experiments with the addition of the injection of acetylcholine during the period of stimulation. As a preliminary acetylcholine was injected in each instance without sympathetic stimulation in order to see whether it produced any action on the capillaries. The only effect observed was a slight slowing of the stream. In some instances it was thought that a slight dilatation took place but this was not constant and when seen was so slight that it was uncertain. The changes caused by sympathetic stimulation in these experiments differed from those described above in that during the normal period the marked pallor due to arteriolar constriction was absent or markedly reduced while the latent period before contraction of the capillaries took place was about doubled. Stimulation during the period of congestion was without effect on the capillaries in which the rate of flow was greatly reduced as was found in the other experiments.

#### DISCUSSION

The results of the present series of experiments confirm those obtained by previous authors that sympathetic stimulation causes contraction of the capillaries independent of its action on the arterioles. Not only was contraction of the capillaries produced in the normal animal but it also took place after the arterioles had been dilated by acetylcholine. They also showed that a profound modification of the action of the sympathetic nervous system on the capillaries takes place during passive congestion. In marked degrees of congestion the capillaries failed to react to sympathetic stimulation and in the lesser degrees the effect was considerably modified. A similar change has been observed by Lewis (16) in man. He found when the pressure in a cuff round the arm was raised more than from

40 to 60 mm Hg he failed to obtain blanching on puncturing adrenalin (1-1000) into the congested area

It is of considerable interest to consider the cause of the altered response during passive congestion. One possibility is that it may be due to the accumulation of acid metabolites, particularly  $\text{CO}_2$  in the vessels and tissue spaces. This supposition was mainly suggested by perfusion experiments in which capillary dilatation was produced by acid solutions of much greater acidity than one would find under living conditions. However Krogh (17) has shown that a considerable increase of  $\text{CO}_2$  in the circulation is without effect on the capillaries while it required the application of very acid mixtures to the frog's tongue to produce any dilatation of the capillaries. As Lewis (16) points out it is improbable that an accumulation in the vessels or tissue spaces of such volatile and weak acids could induce such prolonged effects as these substances would be rapidly removed when normal conditions were restored.

The second possibility that suggests itself is that the profound changes in the capillaries were due to anoxemia. Krogh (17) showed that the changes, which took place in reactive hyperemia following circulatory arrest with complete stasis, were not prevented either in the frog's tongue when it was kept in an atmosphere of pure oxygen so that no cyanosis took place or in the frog's web which was amply supplied with oxygen from the atmosphere. Lewis (16) has demonstrated that this reaction in man takes place after such a short period of stasis as to make it most improbable that it is due to a metabolic change from anoxemia.

The third possibility is that it is due to the accumulation in the tissue spaces during the period of stasis of some substance which acts on the capillaries. Lewis (16) has brought forward strong evidence to show that a non volatile, slowly diffusible substance is constantly produced by the tissues and that it can produce a profound effect on the capillaries when allowed to accumulate. This he terms "H substance" but he shows that it is probably histamine as it resembles the latter so closely in its effects. He has also demonstrated that histamine is capable of preventing or abolishing the response of the vessels to adrenalin. This explanation seems to be the most likely for the changes observed in the present experiments. Obstruc-

tion of the venous flow would permit an accumulation of the dilator substance while the gradual recovery observed would be compatible with the removal of a slowly diffusible product such as the "H-substance"

Although not directly connected with the subject under investigation it was seen that the latent period following sympathetic stimulation before the capillaries contracted was increased when acetylcholine was injected. The changes observed in the capillaries following the injection of acetylcholine when no stimulation of the sympathetic was used were very slight and confirmed the view that the arterioles were mainly responsible for the fall in blood pressure. They did not suggest that the prolonged latent period was directly due to a change in the capillaries. Under normal conditions the arterioles contract after stimulation of the sympathetic and thus prevent fresh blood from entering the capillaries while after acetylcholine the arterioles remain dilated and allow blood to flow freely into the capillaries. Under these circumstances the capillaries would have a greater force to contract against than when a few motionless corpuscles lie in their lumen. Thus the time required for their complete disappearance would become lengthened.

#### CONCLUSIONS

- 1 Peripheral stimulation of the cervical sympathetic caused the capillaries in the rabbit's ear to contract. This effect took place at a definite interval after arteriolar contraction. It was also present when the arterioles were dilated by acetylcholine.

- 2 The latent period between the commencement of stimulation and contraction of the capillaries was prolonged when acetylcholine was injected throughout the time of stimulation.

- 3 The production of congestion by venous obstruction caused either failure of the capillaries to contract as a result of stimulation of the sympathetic nervous system or a considerable prolongation of the latent period depending on the degree of stasis in the individual capillaries.

- 4 It is suggested that individuals with chronic passive congestion show a variation from the normal in their vasomotor reactions, dependent on the degree of stasis.

## BIBLIOGRAPHY

- 1 Beale, L P , Phil Trans Roy Soc. London, 1860, cl, 611 On the Distribution of Nerves to the Elementary Fibres of Striped Muscle
- 2 Glaser, W , Das Vegetative Nervensystem, Müller, Berlin, 1920
- 3 Steinach, E. and Kahn, R H , Archiv f d ges Physiol , 1903, xcvi, 105 Echte contractilität und motorische Innervation der Blutcapillaren
- 4 Krogh, A , Harrop, G A , and Rehberg, P B , J Physiol , 1922, lvi, 179 Studies on the Physiology of Capillaries. III The Innervation of the Blood Vessels in the Hind Legs of the Frog
- 5 Hooker, D R , Physiol. Rev , 1921, i, 112 Evidence of Functional Activity on the Part of the Capillaries and Venules.
- 6 Harris, K. E and Marvin, H M , Heart 1927, xiv, 135 The Innervation of Mammalian Capillaries by Vasoconstrictor Sympathetic Nerves.
- 7 Doi, Y , J Physiol., 1920-21, liv, 227 On the Existence of Antidromic Fibres in the Frog and Their Influence on the Capillaries.
- 8 Langley, J N , J Physiol , 1922-23, lvii, 428 Antidromic Action
- 9 Langley, J N , J Physiol , 1923-24, lviii, 49 Antidromic Action II. Stimulation of the Peripheral Nerves of the Cat's Hind Foot
- 10 Lewis, T , and Marvin, H. M , Heart, 1927, xiv, 27 Observations Relating to Vasodilatation Arising from Antidromic Impulses to Herpes Zoster and Trophic Effects
- 11 Crawford, J H , J Clin Invest , 1926, ii, 365 Studies on Human Capillaries III Observations in Cases of Auricular Fibrillation
- 12 Crawford, J H , J Clin Invest., 1927, iv, 317 Studies on Human Capillaries. V Observations in Cases of Heart Disease with Regular Rhythm
- 13 Hunt, R , and Taveau C , Brit Med. J , 1906, ii, 1788 On the Physiological Action of Certain Cholin Derivatives and New Methods for Detecting Cholin
- 14 Hunt, R., Am J Physiol , 1918, xlv, 197 Vasodilator Reactions I
- 15 Dale, H. H and Richards A. N , J Physiol , 1918-19, lli, 110 The Vasodilator Action of Histamine and of Some Other Substances.
- 16 Lewis T , The Blood Vessels of the Human Skin and Their Responses, London 1927
- 17 Krogh, A The Anatomy and Physiology of Capillaries, New Haven, 1922



# THE EXCRETION OF ZINC IN HEALTH AND DISEASE

By LAWRENCE T. FAIRHALL AND LYMAN H. HOYT

*(From the Department of Physiology Harvard School of Public Health and the Medical Clinic  
Peter Bent Brigham Hospital Boston)*

(Received for publication April 12 1929)

During recent years increasing attention has been directed towards the occurrence of zinc as a normal constituent of animal tissues and fluids (1). Its wide distribution in foodstuffs and particularly its concentration in germ cells—the endosperm of wheat and the yolk of eggs, for example—and constant presence in milk points to a definite utilization. The fact that there is a more or less definite ratio of zinc to calcium in blood in the proportion of 7:100, and that zinc metabolism in animals has been shown to bear some relation to inorganic salt metabolism in general (2), leads to speculation as to the part it plays in the human organism.

For this reason it was felt desirable to secure further data regarding the excretion of absorbed zinc under conditions of disease as compared with the normal. It is interesting to note that man ingests in his food practically as much zinc as he does iron. Far the greater part is excreted through the intestine. A small amount, however, is absorbed and excreted through the urine and, as the latter is a better index of absorbed zinc, a series of analyses of urinary zinc under conditions of health and disease was carried out.

## EXPERIMENTAL

Twenty-four hour specimens of urine were collected from a number of healthy individuals and also from hospital patients suffering from various diseases such as nephritis, uremia, duodenal ulcer, tuberculosis and diabetes. In order to avoid incidental contamination with zinc from glassware, the urine was collected in Pyrex glass-stoppered bottles and evaporated to dryness and ashed in Pyrex dishes. The zinc was separated and determined by a turbidimetric method as the ferrocyanide (3).



# THE EXCRETION OF ZINC IN HEALTH AND DISEASE

By LAWRENCE T. FAIRHALL AND LYMAN H. HOYT

*(From the Department of Physiology Harvard School of Public Health, and the Medical Clinic, Peter Bent Brigham Hospital Boston)*

(Received for publication April 12, 1929)

During recent years increasing attention has been directed towards the occurrence of zinc as a normal constituent of animal tissues and fluids (1). Its wide distribution in foodstuffs and particularly its concentration in germ cells—the endosperm of wheat and the yolk of eggs for example—and constant presence in milk points to a definite utilization. The fact that there is a more or less definite ratio of zinc to calcium in blood in the proportion of 7:100, and that zinc metabolism in animals has been shown to bear some relation to inorganic salt metabolism in general (2), leads to speculation as to the part it plays in the human organism.

For this reason it was felt desirable to secure further data regarding the excretion of absorbed zinc under conditions of disease as compared with the normal. It is interesting to note that man ingests in his food practically as much zinc as he does iron. Far the greater part is excreted through the intestine. A small amount, however, is absorbed and excreted through the urine and, as the latter is a better index of absorbed zinc, a series of analyses of urinary zinc under conditions of health and disease was carried out.

## EXPERIMENTAL

Twenty-four hour specimens of urine were collected from a number of healthy individuals and also from hospital patients suffering from various diseases such as nephritis, uremia, duodenal ulcer, tuberculosis and diabetes. In order to avoid incidental contamination with zinc from glassware, the urine was collected in Pyrex glass stoppered bottles and evaporated to dryness and ashed in Pyrex dishes. The zinc was separated and determined by a turbidimetric method as the ferrocyanide (3).



# THE EXCRETION OF ZINC IN HEALTH AND DISEASE

By LAWRENCE T. FAIRHALL AND LYMAN H. HOYT

*(From the Department of Physiology, Harvard School of Public Health and the Medical Clinic,  
Peter Bent Brigham Hospital, Boston)*

(Received for publication April 12 1929)

During recent years increasing attention has been directed towards the occurrence of zinc as a normal constituent of animal tissues and fluids (1). Its wide distribution in foodstuffs and particularly its concentration in germ cells—the endosperm of wheat and the yolk of eggs, for example—and constant presence in milk points to a definite utilization. The fact that there is a more or less definite ratio of zinc to calcium in blood in the proportion of 7:100, and that zinc metabolism in animals has been shown to bear some relation to inorganic salt metabolism in general (2), leads to speculation as to the part it plays in the human organism.

For this reason it was felt desirable to secure further data regarding the excretion of absorbed zinc under conditions of disease as compared with the normal. It is interesting to note that man ingests in his food practically as much zinc as he does iron. Far the greater part is excreted through the intestine. A small amount, however, is absorbed and excreted through the urine and, as the latter is a better index of absorbed zinc, a series of analyses of urinary zinc under conditions of health and disease was carried out.

## EXPERIMENTAL

Twenty-four hour specimens of urine were collected from a number of healthy individuals and also from hospital patients suffering from various diseases such as nephritis, uremia, duodenal ulcer, tuberculosis and diabetes. In order to avoid incidental contamination with zinc from glassware, the urine was collected in Pyrex glass stoppered bottles and evaporated to dryness and ashed in Pyrex dishes. The zinc was separated and determined by a turbidimetric method as the ferrocyanide (3).

Various clinical pathological data were collected also for comparison with the zinc data. Blood studies included blood urea nitrogen determinations, red blood cell counts, white cell counts and hemoglobins. The presence or absence of edema was noted. Blood pressures and the degree of existing vascular disease were also recorded.

TABLE 1  
*Normal urinary excretion of zinc in health*

| Subject | Urine volume<br>(24 hours) | Total zinc per day | Zinc per liter per day |
|---------|----------------------------|--------------------|------------------------|
|         | <i>cc</i>                  | <i>mgm</i>         | <i>mgm</i>             |
| 1       | 1,200                      | 0 48               | 0 40                   |
| 2       | 1,487                      | 0 69               | 0 46                   |
| 3       | 1,300                      | 0 41               | 0 31                   |
| 4       | 1,610                      | 0 41               | 0 26                   |
| 5       | 1,320                      | 0 28               | 0 21                   |
| 6       | 895                        | 1 00               | 1 12                   |
| 7       | 1,075                      | 0 27               | 0 25                   |
| 8       | 2,260                      | 0 41               | 0 18                   |
| 9       | 1,022                      | 0 59               | 0 58                   |
| 10      | 1,312                      | 0 90               | 0 68                   |
| 11      | 1,140                      | 0 69               | 0 60                   |
| 12      | 795                        | 1 12               | 1 41                   |
| 13      | 975                        | 0 91               | 0 93                   |
| 14      | 626                        | 0 27               | 0 43                   |
| 15      | 1,390                      | 0 27               | 0 19                   |
| 16      | 1,022                      | 1 18               | 1 15                   |
| 17      | 540                        | 0 69               | 1 28                   |
| 18      | 2,092                      | 1 12               | 0 54                   |
| 19      | 1,011                      | 0 34               | 0 34                   |
| 20      | 1,724                      | 1 00               | 0 58                   |
| 21      | 310                        | 0 27               | 0 87                   |
| 22      | 1,575                      | 0 80               | 0 51                   |
| 23      | 836                        | 0 60               | 0 72                   |
| 24      | 1,340                      | 0 45               | 0 34                   |
| Average | 1,202                      | 0 64               | 0 60                   |

Urine examinations for albumin and casts were made. Owing to the fact that none of these figures show any significant correlation with the zinc output they have not been included in the tabulated data. The normal zinc content of the urine of the twenty-four healthy individuals is shown in table 1, while the zinc content of the urine of the patients studied is shown in table 2.

TABLE 2  
*Urinary excretion of zinc in disease*

| Subject  | Type of disease | Urine volume | Total zinc per day | Zinc per liter per day |
|----------|-----------------|--------------|--------------------|------------------------|
|          |                 | cc           | mgm.               | mgm.                   |
| 25       | Nephritis       | 310          | 0 17               | 0 55                   |
| 26       | Nephritis       | 305          | 0 75               | 2 45                   |
| 27       | Nephritis       | 321          | 0 40               | 1 25                   |
| 28       | Nephritis       | 1,836        | 1 30               | 0 71                   |
| 29       | Nephritis       | 516          | 0 70               | 1 36                   |
| 30       | Nephritis       | 1,109        | 0 70               | 0 63                   |
| 31       | Nephritis       | 588          | 0 25               | 0 43                   |
| 32       | Nephritis       | 432          | 0 55               | 1 27                   |
| 33       | Nephritis       | 205          | 0 65               | 3 17                   |
| 34       | Nephritis       | 575          | 0 55               | 0 96                   |
| 35       | Nephritis       | 298          | 0 70               | 2 35                   |
| 36       | Nephritis       | 437          | 0 65               | 1 49                   |
| 37       | Nephritis       | 148          | 0 87               | 5 88                   |
| 38       | Nephritis       | 2,337        | 0 50               | 0 21                   |
| 39       | Nephritis       | 880          | 0 60               | 0 68                   |
| 40       | Nephrosis       | 558          | 0 54               | 0 97                   |
| Average  |                 | 678          | 0 61               | 1 52                   |
| 41       | Uremia          | 1,540        | 1 65               | 1 07                   |
| 42       | Uremia          | 760          | 0 87               | 1 15                   |
| 43       | Uremia          | 1,310        | 1 45               | 1 10                   |
| Average  |                 |              | 1 32               | 1 11                   |
| 44       | Duodenal ulcer  | 1,720        | 1 46               | 0 85                   |
| 45       | Duodenal ulcer  | 1,010        | 0 38               | 0 38                   |
| 46       | Duodenal ulcer  | 1,580        | 1 40               | 0 91                   |
| 47       | Duodenal ulcer  | 1 510        | 0 65               | 0 43                   |
| 48       | Duodenal ulcer  | 1,060        | 0 15               | 0 14                   |
| 49       | Duodenal ulcer  | 950          | 0 45               | 0 47                   |
| 50       | Duodenal ulcer  | 550          | 0 35               | 0 64                   |
| 51       | Duodenal ulcer  | 1 750        | 0 45               | 0 26                   |
| 52       | Duodenal ulcer  | 2,250        | 0 87               | 0 39                   |
| 53       | Duodenal ulcer  | 835          | 1 05               | 1 26                   |
| 54       | Duodenal ulcer  | 2 175        | 0 60               | 0 27                   |
| Average  |                 |              | 0 71               | 0 54                   |
| 55       | Tuberculosis    | 1 985        | 1 55               | 0 78                   |
| 56       | Tuberculosis    | 850          | 0 70               | 0 82                   |
| 57       | Tuberculosis    | 760          | 1 08               | 1 42                   |
| 58       | Tuberculosis    | 1,000        | 1 24               | 1 24                   |
| Average. |                 |              | 1 14               | 1 06                   |

TABLE 2—*Concluded*

| Subject | Type of disease    | Urine volume | Total zinc per day | Zinc per liter per day |
|---------|--------------------|--------------|--------------------|------------------------|
|         |                    | <i>cc</i>    | <i>mgm</i>         | <i>mgm</i>             |
| 59      | Pernicious anemia  | 1,490        | 1 03               | 0 69                   |
| 60      | Pernicious anemia  | 450          | 0 55               | 1 22                   |
| 61      | Lymphatic leukemia | 800          | 0 76               | 0 95                   |
| 62      | Cardiac            | 978          | 0 27               | 0 28                   |
| 63      | Diabetes           | 850          | 0 70               | 0 82                   |
| 64      | Gout               | 1,190        | 1 35               | 1 14                   |
| 65      | Myxedema           | 305          | 0 65               | 2 13                   |

## DISCUSSION

None of the clinical pathological figures obtained from the urea nitrogen determinations, complete blood counts, blood pressure measurements, nor the presence or absence of edema, vascular disease, nor urinary findings were of significance with respect to the zinc output. Nor was the output of zinc greatly altered in cases of disease as compared with the normal amount excreted if the basis of comparison is *total output*. In the case of nephritic patients, the zinc concentration is increased above that of the normal because the total urinary output was small. Whereas the average normal daily output was 0.64 mgm per 24 hours, or 0.60 mgm per liter, with nephritic patients the average total daily output was 0.61 mgm per 24 hours, or 1.52 mgm per liter. Although this is a much higher concentration than normal, it should be noted that the total daily urinary output of the patients with nephritis studied represented but slightly over one-half that of the normal individuals. This was the direct result of the constant restricted low fluid intake which was part of the treatment. If the urinary volume output of the nephritic patients had not been restricted in this manner, there is the possibility that the total zinc excretion figure would have been affected, that is, if the average urine volume of the nephritic patients had been as great as the normal, it is a reasonable assumption that the total zinc output might have been increased in proportion. In any event, in terms of concentration alone, the urinary zinc of the nephritic patients was greater than that of the normal individuals.

Patients with duodenal ulcer show no great departure from the

average for normal individuals. The average total daily excretion of the ulcer cases was 0.71 or 0.54 mgm per liter per day.

In uremia and tuberculosis, the zinc content was distinctly higher than the average normal in every case examined. The average daily excretion of tuberculous patients was 1.14 or 1.06 mgm per liter, while in uremia, the average daily excretion was 1.32 or 1.11 mgm per liter. In these last two cases, therefore, the average zinc excretion was practically double the average normal excretion. Whether these differences are great enough to be of significance is a debatable question. With this as a clue, however, it might prove of interest to investigate the zinc metabolism of tuberculous patients more closely.

#### SUMMARY

An investigation of the urinary zinc excretion of twenty-four healthy individuals and of forty-one hospital patients showed that while no constant relation could be discovered between zinc output and the degree of anemia, or alteration in hemoglobin, blood cell count, urinary albumin or edema, the zinc output is somewhat different in nephritis and markedly accentuated in uremia and tuberculosis.

#### BIBLIOGRAPHY

1. Drinker, K. R., and Collier, E. S., *J. Ind. Hyg.*, 1926, viii, 257. The Significance of Zinc in the Living Organism.  
Lutz, R. E., *J. Ind. Hyg.*, 1926, viii, 177. The Normal Occurrence of Zinc in Biological Materials.  
Birckner, V., *J. Biol. Chem.*, 1919, xxxviii, 191. The Zinc Content of Some Food Products.  
Rost, E., *Die Umschau*, 1920, xxiv, 201. Zinc and Copper, Regular Constituents of the Human Body. (Abstr. in *Chem. Abstr.*, 1929, xiv, 2034.)  
Weitzel, A., *Archiv d. Reichsgesundheitsamtes*, 1918, li, 476. Beiträge zur Bestimmung von Zink in organischen Stoffen.  
Weitzel, A., *Zentralbl. f. Physiol.*, 1914, xxviii, 766. Über das natürliche Vorkommen von Zink in Lebensmitteln und in den Ausscheidungen des Menschen.
2. Fairhall, L. T., *J. Biol. Chem.*, 1926, lxx, 495. The Nutritative Value of Inorganic Substances. I. A Study of the Normal Zinc Metabolism with Particular Reference to the Calcium Metabolism.
3. Fairhall, L. T., *J. Ind. Hyg.*, 1926, viii, 165. The Estimation of Zinc in Biological Material.



## SKIN REACTIONS TO FILTRATES OF HAEMOLYTIC STREPTOCOCCI IN ACUTE AND SUBACUTE NEPHRITIS<sup>1</sup>

BY OSCAR C. HANSEN PRUSS, WARFIELD T LONGCOPE, AND D P O BRIEN<sup>2</sup>  
(From the Medical Clinic, the School of Medicine, Johns Hopkins University, Baltimore)

(Received for publication April 24, 1929)

In a previous communication (1) it was noted that haemolytic streptococci isolated from the tonsils, adenoids or accessory nasal sinuses of cases of acute and subacute glomerular nephritis produced, in broth cultures, "toxins" which caused skin reactions similar to those caused by the "Dick Toxin." While studying the property of these filtrates, it was observed that intense skin reactions often occurred in patients suffering from acute and subacute nephritis associated with infections caused by haemolytic streptococci. A systematic study was, therefore, made of the incidence and intensity of the skin reactions to these bouillon filtrates of haemolytic streptococci in three groups of cases, first, patients suffering from acute and subacute nephritis, secondly, normal individuals and patients affected with miscellaneous conditions, and thirdly, patients suffering from uncomplicated acute tonsillitis. There will be no attempt to discuss at this time the nature of these filtrates.

It is, however, to be noted that many observers have found that haemolytic streptococci recovered from various forms of infection are capable of elaborating "toxic" substances when grown in broth (2, 3, 4, 5, 6, 7, 8, 9, 10, 11).

Although these filtrates are usually more resistant to heat and less potent than the "Dick Toxin," yet they possess other properties in common with the "Dick Toxin," for they are frequently neutralizable by antiscarlatinal serum (3, 7, 9, 10) and are considered by some to differ only quantitatively from the "Dick Toxin," though qualitative differences have also been observed (10, 11, 13, 13a).

<sup>1</sup> Aided by a grant from the Ella Sachs Plotz Foundation.

<sup>2</sup> Jacques Loeb Fellow in Medicine.

The significance of the Dick reaction and of the reactions caused by filtrates obtained from the growth of non-scarlatinal strains of haemolytic streptococci is not perfectly clear. Mackenzie and Hanger (5) considered that the reactions which they obtained indicated an acquired allergy, and that the skin reaction was probably similar in nature to the local tuberculin reaction. There is increasing evidence to sustain this point of view. Dochez and Sherman (12) have shown that the skin of rabbits and guinea pigs, previously inoculated with living cultures of *Streptococcus scarlatinae*, becomes sensitive to filtrates of this organism, while Zinsser and Grinnell (13) have observed that the pronounced skin reactions to haemolytic streptococcus filtrates and to the "Dick Toxin," which can be obtained in guinea pigs infected with haemolytic streptococci, may fade or disappear after the infection has persisted for some days, and later reappear again. They conclude that these reactions are allergic in nature and comparable to the tuberculin reaction. We have observed the same phenomena in rabbits infected subcutaneously by strains of haemolytic streptococci obtained from infections in acute nephritis. The skin of the rabbit which, under normal conditions gives no reaction to the intracutaneous injection of 0.2 cc. of undiluted filtrate, may react to the same filtrates within five to seven days after the onset of an induced local streptococcus infection, by an area of erythema and oedema 1-2 cm. in diameter. Two or three weeks later the hypersensitiveness of the skin usually disappears. We could not determine that these reactions were specific for different strains. Infection of the rabbit by one strain of haemolytic streptococcus rendered the skin sensitive to filtrates from many strains of haemolytic streptococci as well as to the "Dick Toxin." Dochez and Stevens (14) have shown that two phases of cutaneous allergy develop when rabbits are immunized with filtrates of haemolytic streptococci from erysipelas. The reactions occurring during the first period of allergy can be neutralized with erysipelas immune serum, but the reaction during the second phase cannot.

The important observations of Cooke (15), upon the nature of the Dick reaction, bring strong evidence to show that the Dick reaction depends upon an allergy acquired, through streptococcus infections, during early life. A positive reaction may become temporarily

negative through desensitization or be rendered permanently negative by the production in the body of antitoxin. Kirchner (16) states that he can sensitize the cornea of rabbits against filtrates of both scarlatinal strains and non scarlatinal strains of haemolytic streptococci.

#### METHODS

In the present investigation 18 strains of hemolytic streptococci were employed. Thirteen of them were of the beta type and five of alpha type. The organisms were grown in beef infusion peptone broth of pH 7.4 for 18 hours at 36.5°C, which was then filtered through Berkefeld candles and Seitz filters. For skin reactions the filtrates were usually employed in 1-100 dilutions. When positive skin reactions were obtained with these dilutions, tests were made as a rule with 1-500, 1-1000, 1-2000 and occasionally 1-5000 dilutions. For each test 0.1 cc. of the dilution was employed. Stock bouillon in 1-100 dilution was employed as a control and a Dick reaction with 1 S.T.D. of "toxin" was always made at the same time. The skin reactions were read at the end of 18 hours and 24 hours. The reaction was considered negative unless the area of erythema measured more than 1.0 cm. in one diameter. Reactions of 1 to 1.5 cm. were termed +, reactions of 1.5 to 2.0 cm. were termed ++, reactions of 2.0 to 2.5 cm. in diameter were termed +++ and all reactions 2.5 cm. in diameter or over were designated ++++.

Since the observations have been carried on over several years it has been necessary to prepare many batches of filtrates. These have been titrated against a known reactor before they were employed for tests. It was surprising to note with what regularity a single strain produced a filtrate of approximately the same strength.

#### *Skin reactions in normal persons and patients suffering from miscellaneous conditions*

Mackenzie and Hanger (5) found young children quite regularly insensitive to the filtrates which they employed, but obtained positive reactions in a very large proportion of adults. They were not able to correlate these reactions with previous or existing infections, nor could they find any apparent relation between the presence of these skin reactions and any one disease or group of diseases. They employed comparatively large doses of filtrate using 0.03 to 0.04 cc. of undiluted broth filtrates. Howell and Corrigan (17) found that a high percentage of persons, both healthy and diseased, reacted positively to streptococcus filtrates, and that there was no apparent correlation between the skin reactions and the etiological factors of the

disease Children that were susceptible to streptococcus infections gave a higher percentage of positive reactions with all streptococcus filtrates than did other children or adults They suggest that a positive skin reaction with streptococcus filtrates may indicate susceptibility to streptococcus infections Lash and Kaplan (2) obtained 81 per cent positive skin reactions in 247 women who were either normal (20), pregnant (86), or puerperal (141), with filtrates in 1-1000 dilution of a streptococcus obtained from a case of puerperal septicaemia Birkhaug (6) found that 21 per cent of school children, between the ages of 7 and 17 years, gave positive skin reactions to the toxin from *Streptococcus erysipelatis*, while 27 per cent of 135 hospital patients, between the ages of 17 and 72 years, gave positive reactions to 1 S T D

Amoss, Hansen-Pruss and Bluss (18) state that 70 per cent of normal men, and 80 per cent of normal women give more or less marked skin reactions to erysipelas toxin in 1-100 dilution

Statistics concerning the occurrence of positive Dick reactions in children and adults vary considerably Dick and Dick (19) give 58.8 per cent of positive reactions in 1250 persons, Lees (20) 49.8 per cent positive reactions amongst 530 University students, Zingher (21) 26.3 per cent positive reactions amongst children in New York City while Dyer (22) found that school children from rural and urban districts might show a variation in positive reactions from 100 per cent to 25 per cent

It is obviously impossible to make accurate comparisons of the incidence of positive skin reactions to streptococcus filtrates obtained by different observers So much depends upon the dosage, the strength of the various filtrates and the group of individuals tested It is possible, however, to compare with a considerable degree of accuracy the tests performed with standardized filtrates by a single observer in different groups of patients

We have tested the skin of 60 individuals, who were supposedly normal or who were suffering from a miscellany of conditions, with bouillon filtrates of the strains of haemolytic streptococci isolated from infections in acute nephritis Eighteen different strains were employed in the preparation of these filtrates, and the filtrates from 2 to 16 strains were employed for tests on each individual An average

of 9 filtrates was used for tests in each person. Most of these persons were between 18 and 35 years of age. Of the 60 individuals one half were supposedly normal, the other half, though not acutely ill, were affected with a variety of conditions, such as chronic valvular heart disease, duodenal ulcer, multiple sclerosis, hyperthyroidism, chronic tonsillitis, diabetes, hereditary telangiectasis, chronic sinusitis,

TABLE 1  
*Incidence of reactions Normals*

|          | Total number | + or more |          | ++++   |          | Dick + |          |
|----------|--------------|-----------|----------|--------|----------|--------|----------|
|          |              | Number    | Per cent | Number | Per cent | Number | Per cent |
| Subjects | 60           | 38        | 62.2     | 15     | 25       | 14     | 20.3     |
| Tests    | 542          | 167       | 30.8     | 33     | 6        |        |          |

TABLE 2  
*Reactions with less dilute filtrates Normals*

| Subject | Strain of filtrate | Intensity of reaction at following dilutions of filtrate |      |      |
|---------|--------------------|--|------|------|
|         |                    | 1:100  | 1:50 | 1:20 |
| 1       | a                  | 0  | +++  |      |
|         | b                  | ++   | ++++ |      |
| 2       | a                  |  | 0    | 0    |
|         | b                  |  | 0    | 0    |
| 3       | a                  | 0  | 0    |      |
|         | b                  | +  | ++++ |      |
|         | c                  | 0  | +++  |      |
| 4       | a                  | 0  | +    | ++   |
|         | b                  | 0  | 0    | +    |
|         | c                  | 0  | 0    |      |
|         | d                  | +  | +    | +++  |

arteriosclerosis, chronic tuberculosis, convalescence from pneumonia and typhoid fever.

Table 1 gives the incidences of total reactions, the incidence of strong reactions and the proportions of positive reactions in this group of control cases.

It was found that about two thirds of all these patients gave some

reaction to one or more of the filtrates in 1-100 dilutions, but that only one-fourth of the patients gave strong reactions in this dilution. Comparatively few patients gave reactions to dilutions of 1-500 or more. Of 33 individuals, selected largely from those who had reacted to the filtrates in 1-100 dilutions, who were tested with a 1-500 dilution of the filtrates 15 or 45.4 per cent gave + or greater reactions, while only 3 or 9 per cent gave ++++ reactions.

Four of the entire number of the negative reactors were tested with less dilute filtrates, 1-50 and 1-20 being employed. Two individuals who gave negative reactions with 1-100 dilutions showed +++ reactions with 1-50 dilutions. One individual gave a negative reaction at 1-50 but a + reaction at 1-20, and one individual gave negative reactions both with 1-50 and 1-20 dilutions. The titrations of these reactions are given in Table 2.

The positive Dick reactions were obtained almost entirely among the individuals that gave positive reactions to the bouillon filtrates. Of the 14 positive Dick reactions 13 occurred amongst the positive reactors and one amongst the negative reactors.

It is important to draw attention to the fact, that, in the miscellaneous group, there were five individuals who suffered from chronic sinusitis or chronic tonsillitis. All five of these patients gave positive reactions and four of them showed ++++ reactions to one or more of the filtrates. Furthermore all of these five individuals came in the group of 15 who gave + reactions in 1-500 dilutions, while the 3 individuals who gave ++++ reactions in 1-500 dilutions belong to this group of chronic tonsillitis and sinusitis.

From the observations made on these 60 persons, it may be concluded that the skin reactions to these bouillon filtrates of haemolytic streptococci vary considerably in different individuals. The skin of some individuals may be almost insensitive to comparatively large doses of the filtrates, while the skin of other persons may react quite vigorously to considerably diluted filtrate. It was also observed that reactions to the same filtrates might vary from time to time. In several individuals reactions that were at first ++ to ++++ became on subsequent tests +. It was also noted that in this control group the individuals having obvious chronic tonsillitis or sinusitis were among those most likely to give strong reactions.

*Skin reactions in acute and subacute nephritis*

The results obtained from the skin tests made upon patients suffering with acute and subacute nephritis differed, in several respects, from those in the control groups

Twenty-seven patients were tested. An average of 9 filtrates from different strains was used in each patient. Table 3 shows the number and percentage of positive reactions obtained in these twenty seven patients with 0.1 cc. of 1-100 dilutions of filtrates.

It may be seen from the table that this group of patients suffering from nephritis gave reactions much more frequently than the control groups, that they gave many more ++++ reactions, and that they reacted to a greater number of filtrates. When one measures the susceptibility of these patients to the streptococcus filtrates, by titrat-

TABLE 3  
*Incidence of reactions Nephritis*

|          | Total number | + or more |          | ++++   |          | Dick + |          |
|----------|--------------|-----------|----------|--------|----------|--------|----------|
|          |              | Number    | Per cent | Number | Per cent | Number | Per cent |
| Patients | 27           | 22        | 81.4     | 18     | 66.6     | 9      | 33.3     |
| Tests    | 259          | 162       | 62.5     | 62     | 23.9     |        |          |

ing the dose of filtrate, the difference becomes even more striking. Of 12 patients tested with 1-500 dilutions 9, or 75 per cent gave + to ++++ reactions, whereas in the control group only 45.4 per cent of 33 cases tested with 1-500 dilutions gave + reactions. Of the 17 nephritics 6, or 50 per cent, gave ++++ reactions with 1-500 dilutions whereas only 9 per cent of the controls gave ++++ reactions with this dilution. In the cases of nephritis, many positive reactions were obtained with still greater dilution, of filtrate. Of 7 patients tested with 1-1000 dilution, 5 gave + reactions or greater and 2 ++++ reactions. Of 5 patients tested with 1-2000 dilutions, 2 gave ++++ reactions, and one of two patients tested with 1-5000 dilutions gave a + reaction.

In the group of nephritics, there seemed to be no definite relationship between reactions from filtrates and reactions from "Dick Toxin." There were 9 of the 27 patients who gave positive Dick reactions. All

of these gave + + + + reactions with the bouillon filtrates. Of the 18 patients who gave negative Dick reactions 9 or 50 per cent gave + + + + reactions with the bouillon filtrates. It can only be said, therefore, that when the Dick reaction was positive the reactions with filtrate were strongly positive, but that strongly positive reactions with filtrates occurred in one-half the patients that gave negative Dick reactions.

Amongst the 27 nephritics, there were 4 cases that gave no reactions to any filtrates of the streptococci, and that also gave negative Dick reactions. Three of these patients had marked oedema at the time the tests were made. One of these three, who had much oedema at the time the first tests were made, was retested later after the oedema had subsided and still showed completely negative reactions. The fourth had only moderate oedema. It is possible that marked oedema of the subcutaneous tissues may favor rapid absorption of the injected bacterial filtrate, such as occurs with sodium chloride solution, and thereby reduce the liability of a reaction in the skin, though it is more probable that the skin of these patients was actually insensitive to the filtrates.

The skin reactions in this group of 27 nephritics seemed to persist in the same degree and intensity in many patients for months or even years. Two patients have shown skin reactions of the same intensity for 8 months, 2 for 1 year, 1 for 1 year and 8 months, one for 2 years, 2 for 2 years and 8 months, and one for 3 years. Among this group of 9 retested patients, there are three who have, apparently, recovered completely from their attacks of acute nephritis as well as from the infection. The acute nephritis in each instance was associated with an acute tonsillitis due to  $\beta$  haemolytic streptococci. In all three, tonsillectomy was performed during the early stage of nephritis. Repeated examinations over a period of 8 months in one case and 2 years and 8 months in the other two have shown that they were free from infection, and cultures from the pharynx and nasopharynx have not shown  $\beta$  haemolytic streptococci. Three of the 9 patients retested are symptomatically well, but still show traces of albumen and occasional hyaline casts in the urine, while cultures from the pharynx have shown from time to time haemolytic streptococci, in the remaining group of three of the total 9 cases, the original acute or subacute

nephritis has become chronic, infections have persisted and cultures from the infections of the pharynx and paranasal sinuses have shown constantly haemolytic streptococci

These observations indicate that the increased sensitiveness of the skin of patients, suffering from acute and subacute nephritis, may persist for long periods of time, and that skin reactions of great intensity, in these patients, are not necessarily dependent upon the existence of a demonstrable infection or upon the carrier state

It seemed necessary, however, to obtain further information as to the relation of the positive skin reaction to acute infections, produced by haemolytic streptococci of  $\beta$  type, occurring in patients in whom there was no evidence of a complicating acute nephritis For this purpose, cases of uncomplicated acute tonsillitis were selected Skin

TABLE 4  
*Incidence of reactions Tonsillitis*

|          | Total number | + or more |          | ++++   |          | Dick + |          |
|----------|--------------|-----------|----------|--------|----------|--------|----------|
|          |              | Number    | Per cent | Number | Per cent | Number | Per cent |
| Subjects | 22           | 21        | 95.4     | 4      | 18.1     | 11     | 50       |
| Tests    | 123          | 70        | 56.9     | 4      | 3.2      |        |          |

reactions were performed with the bouillon filtrates in 22 such cases Haemolytic streptococci were obtained in cultures from the tonsils in all cases An average of five filtrates was employed for the tests in each case. In all but four, the filtrates were used in 1-500 dilutions, and therefore the results are not exactly comparable to those obtained in the control groups The table 4, however, shows the incidence of positive reactions in this group of uncomplicated tonsillitis

It will be seen that though a very large percentage of these cases gave positive reactions, a comparatively small number showed strongly positive reactions in 1-500 dilution, in the tonsillitis group 18.1 per cent and in the nephritis group 50 per cent. Though the total number of cases is small, the pronounced variations are indicative of considerable difference in the sensitiveness of the three groups to the bouillon filtrates

of these gave + + + + reactions with the bouillon filtrates. Of the 18 patients who gave negative Dick reactions 9 or 50 per cent gave + + + + reactions with the bouillon filtrates. It can only be said, therefore, that when the Dick reaction was positive the reactions with filtrate were strongly positive, but that strongly positive reactions with filtrates occurred in one-half the patients that gave negative Dick reactions.

Amongst the 27 nephritics, there were 4 cases that gave no reactions to any filtrates of the streptococci, and that also gave negative Dick reactions. Three of these patients had marked oedema at the time the tests were made. One of these three, who had much oedema at the time the first tests were made, was retested later after the oedema had subsided and still showed completely negative reactions. The fourth had only moderate oedema. It is possible that marked oedema of the subcutaneous tissues may favor rapid absorption of the injected bacterial filtrate, such as occurs with sodium chloride solution, and thereby reduce the liability of a reaction in the skin, though it is more probable that the skin of these patients was actually insensitive to the filtrates.

The skin reactions in this group of 27 nephritics seemed to persist in the same degree and intensity in many patients for months or even years. Two patients have shown skin reactions of the same intensity for 8 months, 2 for 1 year, 1 for 1 year and 8 months, one for 2 years, 2 for 2 years and 8 months, and one for 3 years. Among this group of 9 retested patients, there are three who have, apparently, recovered completely from their attacks of acute nephritis as well as from the infection. The acute nephritis in each instance was associated with an acute tonsillitis due to  $\beta$  haemolytic streptococci. In all three, tonsillectomy was performed during the early stage of nephritis. Repeated examinations over a period of 8 months in one case and 2 years and 8 months in the other two have shown that they were free from infection, and cultures from the pharynx and nasopharynx have not shown  $\beta$  haemolytic streptococci. Three of the 9 patients retested are symptomatically well, but still show traces of albumen and occasional hyaline casts in the urine, while cultures from the pharynx have shown from time to time haemolytic streptococci, in the remaining group of three of the total 9 cases, the original acute or subacute

nephritis has become chronic, infections have persisted and cultures from the infections of the pharynx and paranasal sinuses have shown constantly haemolytic streptococci

These observations indicate that the increased sensitiveness of the skin of patients, suffering from acute and subacute nephritis, may persist for long periods of time, and that skin reactions of great intensity, in these patients, are not necessarily dependent upon the existence of a demonstrable infection or upon the carrier state

It seemed necessary, however, to obtain further information as to the relation of the positive skin reaction to acute infections, produced by haemolytic streptococci of  $\beta$  type, occurring in patients in whom there was no evidence of a complicating acute nephritis. For this purpose, cases of uncomplicated acute tonsillitis were selected. Skin

TABLE 4  
*Incidence of reactions Tonsillitis*

|          | Total<br>number | + or more |          | ++++   |          | Dick + |          |
|----------|-----------------|-----------|----------|--------|----------|--------|----------|
|          |                 | Number    | Per cent | Number | Per cent | Number | Per cent |
| Subjects | 22              | 21        | 95.4     | 4      | 18.1     | 11     | 50       |
| Tests    | 123             | 70        | 56.9     | 4      | 3.2      |        |          |

reactions were performed with the bouillon filtrates in 22 such cases. Haemolytic streptococci were obtained in cultures from the tonsils in all cases. An average of five filtrates was employed for the tests in each case. In all but four, the filtrates were used in 1-500 dilutions, and therefore the results are not exactly comparable to those obtained in the control groups. The table 4, however, shows the incidence of positive reactions in this group of uncomplicated tonsillitis.

It will be seen that though a very large percentage of these cases gave positive reactions, a comparatively small number showed strongly positive reactions in 1-500 dilution, in the tonsillitis group 18.1 per cent and in the nephritis group 50 per cent. Though the total number of cases is small, the pronounced variations are indicative of considerable difference in the sensitiveness of the three groups to the bouillon filtrates.

of these gave + + + + reactions with the bouillon filtrates. Of the 18 patients who gave negative Dick reactions 9 or 50 per cent gave + + + + reactions with the bouillon filtrates. It can only be said, therefore, that when the Dick reaction was positive the reactions with filtrate were strongly positive, but that strongly positive reactions with filtrates occurred in one-half the patients that gave negative Dick reactions.

Amongst the 27 nephritics, there were 4 cases that gave no reactions to any filtrates of the streptococci, and that also gave negative Dick reactions. Three of these patients had marked oedema at the time the tests were made. One of these three, who had much oedema at the time the first tests were made, was retested later after the oedema had subsided and still showed completely negative reactions. The fourth had only moderate oedema. It is possible that marked oedema of the subcutaneous tissues may favor rapid absorption of the injected bacterial filtrate, such as occurs with sodium chloride solution, and thereby reduce the liability of a reaction in the skin, though it is more probable that the skin of these patients was actually insensitive to the filtrates.

The skin reactions in this group of 27 nephritics seemed to persist in the same degree and intensity in many patients for months or even years. Two patients have shown skin reactions of the same intensity for 8 months, 2 for 1 year, 1 for 1 year and 8 months, one for 2 years, 2 for 2 years and 8 months, and one for 3 years. Among this group of 9 retested patients, there are three who have, apparently, recovered completely from their attacks of acute nephritis as well as from the infection. The acute nephritis in each instance was associated with an acute tonsillitis due to  $\beta$  haemolytic streptococci. In all three, tonsillectomy was performed during the early stage of nephritis. Repeated examinations over a period of 8 months in one case and 2 years and 8 months in the other two have shown that they were free from infection, and cultures from the pharynx and nasopharynx have not shown  $\beta$  haemolytic streptococci. Three of the 9 patients retested are symptomatically well, but still show traces of albumen and occasional hyaline casts in the urine, while cultures from the pharynx have shown from time to time haemolytic streptococci, in the remaining group of three of the total 9 cases, the original acute or subacute

the preponderance of strongly positive skin reactions in the nephritic group, for the nephritics continued to show this peculiarity, in several instances, for months or years after the streptococcus infection had disappeared. Moreover, if one regards the results from another point of view, it appears that a fair proportion of all the individuals who gave strongly positive skin reactions were nephritic. Of the total number of 109 individuals tested, 37 or 33.9 per cent gave strongly positive reactions, and of these 37 strongly positive reactors, 18 or 48.6 per cent were nephritic.

There appears, actually, to be a quantitative difference in the skin reactions to these filtrates, between many patients with nephritis and the average normal individual, or the patient suffering from an uncomplicated acute streptococcus tonsillitis. This difference consists in a heightened susceptibility of the skin to the culture filtrates of haemolytic streptococci. It seems possible, that there may be some connection between the heightened susceptibility of the skin to these filtrates, and the occurrence of acute glomerular nephritis as a complication of local streptococcus infection. It is becoming more and more obvious that in the streptococcus infections, as in tuberculosis and syphilis, an induced allergy, towards the protein of the streptococcus or to the products of its growth, accounts for some of the variations in the form of the infection and of its complications. Bristol (23) has expressed the view that the exanthem of scarlet fever is an allergic reaction to the local streptococcus infection, and has reviewed the literature on this subject. The more recent investigations by Dochez and Stevens (14) and by Cooke (15) go far to substantiate this idea. Dochez and Stevens conclude that both the Dick reaction and the rash in scarlet fever are dependent upon previous sensitization of the individual to haemolytic streptococci, and are to be looked upon as allergic reactions of the individual to products of *Streptococcus scarlatinae*. Cooke adds considerable evidence to confirm this hypothesis in which he thoroughly concurs.

Another streptococcus infection, which presents certain features that have been interpreted as allergic in nature, is erysipelas. Amoss, Hansen Prüss and Bliss (18) bring evidence to show that recurrent attacks of erysipelas are allergic in character, and find that the skin reactions to bouillon filtrates of erysipelas strains of haemolytic

## DISCUSSION

A study of the skin tests made with filtrates of broth cultures of haemolytic streptococci, in these three groups of cases, shows the necessity, in any comparative investigation, of using different dilutions of the filtrate to distinguish varying degrees of reactivity of the skin. Amongst the individuals in the three groups, one finds, by titrating the reaction, all transitions from those, who are practically insensitive to the filtrates in the doses employed, to those who react vigorously to 1-2000 dilutions. The control group includes the largest proportion of poor reactors, whereas the nephritic group contains the largest number of strong reactors. The tonsillitis group contains many reactors, but comparatively few strong reactors. Table 5 demonstrates quite clearly these differences.

TABLE 5  
*Summary of incidence of reactions*

| Diagnosis |             | Total number | +      |          | ++++   |          |
|-----------|-------------|--------------|--------|----------|--------|----------|
|           |             |              | Number | Per cent | Number | Per cent |
| Cases     | Controls    | 60           | 38     | 62.2     | 15     | 25       |
|           | Tonsillitis | 22           | 21     | 95.4     | 4      | 18.1     |
|           | Nephritis   | 27           | 22     | 81.4     | 18     | 66.6     |
| Tests     | Normal      | 542          | 167    | 30.8     | 33     | 6.0      |
|           | Tonsillitis | 123          | 70     | 56.9     | 4      | 3.2      |
|           | Nephritis   | 259          | 162    | 62.5     | 62     | 23.9     |

In the nephritic group, not only was the proportion of strongly positive reactions very high, but each positive reactor in the group showed strongly positive reactions to many more strains than the positive reactors in the other groups. Though the total number of cases is not large, yet the differences are so pronounced between the groups, that one is inclined to attribute them to actual variations in the susceptibility to the filtrates rather than to chance. The results obtained, both in the control group and in the tonsillitis group, indicate that persons suffering from various forms of infection due, to  $\beta$  haemolytic streptococci, are somewhat more likely to give positive skin reactions to these streptococcus filtrates than are normal individuals. It does not seem, however, that this propensity can account entirely for

the preponderance of strongly positive skin reactions in the nephritic group, for the nephritics continued to show this peculiarity, in several instances, for months or years after the streptococcus infection had disappeared. Moreover, if one regards the results from another point of view, it appears that a fair proportion of all the individuals who gave strongly positive skin reactions were nephritic. Of the total number of 109 individuals tested, 37 or 33.9 per cent gave strongly positive reactions, and of these 37 strongly positive reactors, 18 or 48.6 per cent were nephritic.

There appears, actually, to be a quantitative difference in the skin reactions to these filtrates, between many patients with nephritis and the average normal individual, or the patient suffering from an uncomplicated acute streptococcus tonsillitis. This difference consists in a heightened susceptibility of the skin to the culture filtrates of haemolytic streptococci. It seems possible, that there may be some connection between the heightened susceptibility of the skin to these filtrates, and the occurrence of acute glomerular nephritis as a complication of local streptococcus infection. It is becoming more and more obvious that in the streptococcus infections, as in tuberculosis and syphilis, an induced allergy, towards the protein of the streptococcus or to the products of its growth, accounts for some of the variations in the form of the infection and of its complications. Bristol (23) has expressed the view that the exanthem of scarlet fever is an allergic reaction to the local streptococcus infection, and has reviewed the literature on this subject. The more recent investigations by Dochez and Stevens (14) and by Cooke (15) go far to substantiate this idea. Dochez and Stevens conclude that both the Dick reaction and the rash in scarlet fever are dependent upon previous sensitization of the individual to haemolytic streptococci, and are to be looked upon as allergic reactions of the individual to products of *Streptococcus scarlatinae*. Cooke adds considerable evidence to confirm this hypothesis in which he thoroughly concurs.

Another streptococcus infection, which presents certain features that have been interpreted as allergic in nature, is erysipelas. Amoss, Hansen Prüss and Bliss (18) bring evidence to show that recurrent attacks of erysipelas are allergic in character, and find that the skin reactions to bouillon filtrates of erysipelas strains of haemolytic

streptococci are pronounced in all patients suffering from recurrent attacks of erysipelas. Birkhaug (24) has expressed a similar view, while Francis (25) concludes, from a study of erysipelas, that allergy plays an important rôle both in the pathogenesis of the disease and in recovery from the local lesion.

Some observations have likewise been made upon the allergic reactions to non-haemolytic streptococci. The work of Birkhaug (26), Kaiser (27) and Swift, Wilson and Todd (28) indicates that skin reactions to various strains of non-haemolytic streptococci, or to their filtrates, are more frequent in rheumatic fever than in non-rheumatic patients, while Swift, Wilson and Todd observed a larger proportion of positive skin reactions, during the active stage of the disease, than during the latent period. Swift, Hitchcock and Derick (29), moreover, have obtained general tuberculin like reactions, occasionally with reactivation of quiescent rheumatic foci, in patients with rheumatic fever who have been injected intravenously with heated killed vaccines of both green and haemolytic streptococci, or with nucleoprotein of haemolytic streptococci. Swift (30), in particular, has done much to further the conception that the arthritis of rheumatic fever is an allergic reaction, the result of preliminary sensitization of the joints to non-haemolytic streptococci, and Zinsser (31) is inclined to accept this hypothesis.

The idea that the inception of acute glomerular nephritis may be dependent upon an allergic reaction of the kidneys is not new, for both Schick (32) and von Pirquet (33) suggested, many years ago, that the acute nephritis of scarlet fever might be interpreted as an allergic manifestation of this disease. An attempt was made by one of us, some time ago, to produce nephritis in animals by repeated anaphylactic shock, (34) and though these experiments, performed with soluble proteins, did result in degenerative lesions in the epithelium of the kidney tubules, with inflammatory reactions occasionally affecting the glomeruli, the pathological picture of diffuse glomerular nephritis was not obtained, and the results do not seem applicable to an explanation of the origin of nephritis in human beings. It seems possible, however, that when the body has become sensitized to an infection, the kidney may respond, in somewhat the same manner as the skin, when the products of bacterial growth are brought in direct contact with the

kidney cells Long and Finner (35) have recently described the typical lesions of diffuse glomerular nephritis produced, in the tuberculous pig, by the injection of tuberculin into the renal artery

If the heightened skin reaction to the filtrates of haemolytic streptococci can be interpreted as allergic reactions, then our observations indicate, that many patients, suffering from acute and subacute nephritis, are quite highly allergic to some constituent of haemolytic streptococci or to substances elaborated by these organisms. Continued investigations upon the incidence of haemolytic streptococcus infection, at the onset, during the course and with exacerbations of acute and subacute glomerular nephritis, have confirmed us in the view that this relationship is too frequent and too close to be purely incidental. Repeated cultures from the urine, even during the early stages of acute nephritis, have not shown haemolytic streptococci, except in the rare instances of what is probably a focal form of glomerular nephritis, and we have not obtained any evidence to show that the diffuse glomerular nephritis is the result of an actual infection of the kidney by bacteria. Though the idea, so frequently suggested, that the diffuse glomerular lesions are produced by the direct action of a true toxin cannot be dismissed, it seems much more probable that the reaction in the kidney may be allergic in nature, similar possibly to the reaction in the skin, and dependent upon a sensitization of the kidney cells to the haemolytic streptococci or the products of their growth.

If such a condition existed, one would scarcely expect absolute correlation between the skin reaction and the kidney reaction, for in other forms of sensitization this does not occur. Most patients suffering from allergic hay fever give skin reactions to the substances causing the hay fever or asthma, but they may, in addition give skin reactions to many substances that are not concerned in the production of their asthma or hay fever. Conversely, the ingestion of a particular kind of food may cause urticaria and gastro-intestinal symptoms in patients whose skins do not react to extracts of this especial food. The correlation, therefore, between the skin reaction and the somatic reaction is not absolutely constant. Should the same conditions hold for streptococcus infections, it would not be surprising to encounter patients who gave strong skin reactions to filtrates of haemolytic streptococci, and yet did not develop nephritis during the course of a

streptococci are pronounced in all patients suffering from recurrent attacks of erysipelas Birkhaug (24) has expressed a similar view, while Francis (25) concludes, from a study of erysipelas, that allergy plays an important rôle both in the pathogenesis of the disease and in recovery from the local lesion

Some observations have likewise been made upon the allergic reactions to non-haemolytic streptococci The work of Birkhaug (26), Kaiser (27) and Swift, Wilson and Todd (28) indicates that skin reactions to various strains of non-haemolytic streptococci, or to their filtrates, are more frequent in rheumatic fever than in non-rheumatic patients, while Swift, Wilson and Todd observed a larger proportion of positive skin reactions, during the active stage of the disease, than during the latent period Swift, Hitchcock and Derick (29), moreover, have obtained general tuberculin like reactions, occasionally with reactivation of quiescent rheumatic foci, in patients with rheumatic fever who have been injected intravenously with heated killed vaccines of both green and haemolytic streptococci, or with nucleo protein of haemolytic streptococci Swift (30), in particular, has done much to further the conception that the arthritis of rheumatic fever is an allergic reaction, the result of preliminary sensitization of the joints to non-haemolytic streptococci, and Zinsser (31) is inclined to accept this hypothesis

The idea that the inception of acute glomerular nephritis may be dependent upon an allergic reaction of the kidneys is not new, for both Schuck (32) and von Pirquet (33) suggested, many years ago, that the acute nephritis of scarlet fever might be interpreted as an allergic manifestation of this disease An attempt was made by one of us, some time ago, to produce nephritis in animals by repeated anaphylactic shock, (34) and though these experiments, performed with soluble proteins, did result in degenerative lesions in the epithelium of the kidney tubules, with inflammatory reactions occasionally affecting the glomeruli, the pathological picture of diffuse glomerular nephritis was not obtained, and the results do not seem applicable to an explanation of the origin of nephritis in human beings It seems possible, however, that when the body has become sensitized to an infection, the kidney may respond, in somewhat the same manner as the skin, when the products of bacterial growth are brought in direct contact with the

## BIBLIOGRAPHY

- 1 Longcope, W T , O'Brien, D P , McGuire, J , Hansen, O C , and Denny, E R., J Clin Invest , 1927, v 7 Relationship of Acute Infections to Glomerular Nephritis.
- 2 Lash, A. F , and Kaplan, B , J Am. Med. Assoc , 1925, lxxxiv, 1991, 1926, lxxxvi, 1197, 1427 Puerperal Fever
- 3 Kirkbride, M B , and Wheeler, M W , J Immunol., 1926, xi, 477, 1927, xii, 19 Studies of the Toxins of the Hemolytic Streptococci Associated with Scarlet Fever
- 4 Teichmann, J Ztschr f Immunitätsforsch , 1926, xlviii, 466 Experimentelle Prüfung des Dick Toxins
- 5 Mackenzie, G M , and Hanger, F M , Jr , J of Immunol., 1927, xii, 41 Allergic Reactions to Streptococcus Antigens
- 6 Birkhaug, K E Proc. Soc. Exp Biol and Med., 1925, xxx, 201, 291 Studies on the Biology of the *Streptococcus erysipelas* IV Toxin Production of the *Streptococcus erysipelas*
- 7 Smith, J , J Path and Bact., 1927, xxx, 651 The Exotoxins of the Haemolytic Streptococci.
- 8 Okell, C C , and Parish, H J , Lancet, 1928, i, 748 The Relationship of Scarlet Fever to Other Streptococcal Infections
- 9 Zlatogoroff, S I., and Derkatch, W S , J Infect. Dis , 1928, xlii, 56 On the Specificity of Scarlet Fever Streptococci.
- 10 Ando, K., Kurauchi, K., and Ozaki, K., J Immunol , 1928, xv, 191, 217 Studies on the "Toxins" of Hemolytic Streptococci. I Relationship of the Toxins of Different Hemolytic Streptococci as Brought Out by Tests on the Human Subjects
- 11 Hirsfeld H , Mayzner, M , and Przesmycki, F , Ztschr f Immunitätsforsch., 1928, lvi, 414 Untersuchungen über Streptokokkengifte
- 12 Dochez, A R., and Sherman, L , Proc. Soc. Exp Biol and Med , 1925, xxii, 282 Some Reactions in Sensitized Guinea Pigs to the Filtrate of Scarlatinal Streptococcus
- 13 Zinsser, H , and Grinnell, F B , J Immunol., 1925, x, 725 Further Studies on Bacterial Allergy Allergic Reactions to the Hemolytic Streptococcus
- 13a. Williams, A. W Am. J Pub Health, 1925, xv, 129 The Relationship Between Different Antibodies
- 14 Dochez A R., and Stevens, F A., J Exp Med., 1927, xlv, 487 Studies on the Biology of Streptococcus VII Allergic Reactions with Strains from Erysipelas
- 15 Cooke, J V Am J Dis Child , 1927, xxxiv, 969 1928, xxxv, 762, 772, 781, 784 974, 983, 991 Scarlet Fever
- 16 Kirchner O Ztschr f Immunitätsforsch 1928, lv, 157 Versuche über die Wirkung der Filtrate von Scharlach-streptokokken und anderen Streptokokken auf die Kaninchenkornea.

streptococcus infection, and, on the other hand, observe occasional instances in which nephritis occurred, during the course of a streptococcus infection, in an individual who did not show exaggerated skin reactions to the filtrate

In the groups of patients which we have studied, however, the most intense and the most numerous skin reactions to filtrates of haemolytic streptococci have occurred in those suffering from acute and subacute nephritis. Though the nephritis occurred, in the majority of instances, in persons suffering from infections caused by haemolytic streptococci, the positive skin reactions themselves, it was found, might persist long after the disappearance of the infection, and when the patient had apparently recovered from nephritis. It was, moreover, found that almost one half of the strongly positive reactors were nephritic.

#### SUMMARY

Skin reactions to the filtrates of haemolytic streptococci were studied in three groups of cases: (1) A control group of normal individuals and patients suffering with a variety of diseases, (2) patients with acute tonsillitis, (3) patients with acute and subacute nephritis.

In the control group 62 per cent were found to give positive skin reactions, but only 25 per cent strongly positive reactions, in the tonsillitis group 95.4 per cent were found to give positive skin reactions, but only 18.1 per cent strongly positive reactions, in the nephritis group, however, 81.4 per cent gave positive skin reactions, and 66.6 per cent strongly positive reactions.

The positive skin reaction is regarded as an evidence of allergy to the haemolytic streptococcus or the products of its growth.

The preponderance of strongly positive reactions in the nephritis group indicates that these patients may be highly allergic to the haemolytic streptococcus or the products of its growth.

It is suggested that the development of acute diffuse glomerular nephritis, in patients suffering from haemolytic streptococcus infections, may be referable to the products of the growth of the haemolytic streptococcus acting upon previously sensitized kidney cells.

## BIBLIOGRAPHY

- 1 Longcope, W T, O'Brien, D P, McGuire, J, Hansen, O C, and Denny, E R., J Clin Invest, 1927, v 7 Relationship of Acute Infections to Glomerular Nephritis
- 2 Lash, A F, and Kaplan, B, J Am Med Assoc., 1925, lxxxiv, 1991, 1926, lxxxvi, 1197, 1427 Puerperal Fever
- 3 Kirkbride, M B, and Wheeler, M W, J Immunol, 1926, xi, 477, 1927, xii, 19 Studies of the Toxins of the Hemolytic Streptococci Associated with Scarlet Fever
- 4 Teichmann, J, Ztschr f Immunitätsforsch, 1926, xlviii, 466 Experimentelle Prüfung des Dick Toxins
- 5 Mackenzie, G M, and Hanger, F M, Jr, J of Immunol, 1927, xii, 41 Allergic Reactions to Streptococcus Antigens
- 6 Birkhaug, K E, Proc. Soc. Exp Biol and Med, 1925, xxii, 201, 291 Studies on the Biology of the *Streptococcus erysipelatis* IV Toxin Production of the *Streptococcus erysipelatis*
- 7 Smith, J, J Path and Bact, 1927, xxx, 651 The Exotoxins of the Haemolytic Streptococci
- 8 Okell, C C, and Parish, H J, Lancet, 1928, i, 748 The Relationship of Scarlet Fever to Other Streptococcal Infections
- 9 Zlatogoroff, S L, and Derkatch, W S, J Infect Dis, 1928, xlii, 56 On the Specificity of Scarlet Fever Streptococci.
- 10 Ando, K, Kurauchi, K., and Ozaki, K., J Immunol., 1928, xv, 191, 217 Studies on the "Toxins" of Hemolytic Streptococci. I Relationship of the Toxins of Different Hemolytic Streptococci as Brought Out by Tests on the Human Subjects
- 11 Hirszfeld, H, Mayzner, M, and Przesmycki, F, Ztschr f Immunitätsforsch, 1928, lvii, 414 Untersuchungen über Streptokokkengifte
- 12 Dochez, A R., and Sherman, L., Proc. Soc. Exp Biol. and Med, 1925, xxii, 282 Some Reactions in Sensitized Guinea Pigs to the Filtrate of Scarlatinal Streptococcus.
- 13 Zinsser, H, and Grinnell, F B, J Immunol., 1925, x, 725 Further Studies on Bacterial Allergy Allergic Reactions to the Hemolytic Streptococcus
- 13a Williams, A W, Am. J Pub Health, 1925, xv, 129 The Relationship Between Different Antibodies
- 14 Dochez, A R, and Stevens F A, J Exp Med., 1927, xlii, 487 Studies on the Biology of Streptococcus VII. Allergic Reactions with Strains from Erysipelas
- 15 Cooke, J V, Am J Dis. Child, 1927, xxxiv, 969, 1928 xxv, 762, 772, 781, 784, 974 983, 991 Scarlet Fever
- 16 Kirchner, O, Ztschr f Immunitätsforsch., 1928 lv, 157 Versuche über die Wirkung der Filtrate von Scharlach-streptokokken und anderen Streptokokken auf die Kaninchenkornea

- 17 Howell, K M , and Corrigan, M , J Infect. Dis , 1928, xlii, 149 Skin Reactions with Bacterial Filtrates of Anhemolytic Streptococcus, Hemolytic Streptococcus and *B typhosus*
- 18 Amoss, H L , Hansen-Prüss, O C , and Bliss, E A , Trans Assoc Am Phys , 1928, xliii, 259 Erysipeloid Reactions as an Allergic Response
- 19 Dick, G F , and Dick, G H , J Am Med Assoc., 1925, lxxxiv, 1477 Results with the Skin Test for Susceptibility to Scarlet Fever
- 20 Lees, H D , J Am Med Assoc , 1927, lxxxviii, 1133 The Dick Test with Active and Passive Immunization for Scarlet Fever
- 21 Zingher, A , J Am Med Assoc , 1924, lxxxiii, 432 The Dick Test in Normal Person and in Acute and Convalescing Cases of Scarlet Fever
- 22 Dyer, R E , Caton, W P , and Sockrider, B T , Public Health Reports, 1926, xli, 1159 Results of Dick Tests Made on Different Groups
- 23 Bristol, L D , Am J Med Sci , 1923, clxvi, 853 Scarlet Fever as a Reaction of Hypersensitiveness to Streptococcus Protein
- 24 Birkhaug, K E , J Am Med Assoc , 1928, xc, 1997 Erysipelas VIII Bacterial Allergy to *Streptococcus erysipelatis* in Recurrent Erysipelas
- 25 Francis, T , Jr J Clin Invest , 1928, vi, 221 Studies on Pathogenesis and Recovery in Erysipelas
- 26 Birkhaug, K E , J Infect Dis , 1927, xl, 549 Rheumatic Fever Bacteriologic Studies of a Non-Methemoglobin-Forming Streptococcus with Special Reference to its Soluble Toxin Production
- 27 Kaiser, A D , J Infect Dis , 1928, xlii, 25 Skin Reactions in Rheumatic Fever
- 28 Swift, H S , Wilson, M G , Todd, E W , Am J Dis Child , 1929, xxxvii, 98 Skin Reactions of Patients with Rheumatic Fever to Toxic Filtrates of Streptococcus
- 29 Swift, H F , Hitchcock, C H , and Derick, C L , Proc Soc Exp Biol and Med , 1928, xxv, 312 General Tuberculin-like Reactions in Rheumatic Fever Patients Following Intravenous Injections of Streptococcus Vaccines or Nucleo-proteins
- 30 Swift, H F , Derick, C L , and Hitchcock, C H , J Am Med Assoc , 1928, xc, 906 Trans Assoc Am Phys , 1928, xliii, 192 Bacterial Allergy (Hyperergy) to Nonhemolytic Streptococci Rheumatic Fever as a Manifestation of Hypersensitiveness (Allergy or Hyperergy) to Streptococci
- 31 Zinsser, H , and Yu, H , Arch Int. Med , 1928, xlii, 301 The Bacteriology of Rheumatic Fever and the Allergic Hypothesis
- 32 Schick, B , Jahrbch f Kinderheilkunde, 1907, lxxv, 132 Die Nachkrankheiten des Scharlach
- 33 von Pirquet, C E , Arch Int Med , 1911, vii, 259, 383 Allergy
- 34 Longcope, W T , J Exp Med , 1913, xviii, 678 The Production of Experimental Nephritis by Repeated Protein Intoxication
- 35 Long, E R , and Finner, L L , Am J Path , 1928, iv, 571 Experimental Glomerular Nephritis Produced by Intrarenal Tuberculin Reactions

# THE COLOR OF THE SKIN AS ANALYZED BY SPECTROPHOTOMETRIC METHODS

## I APPARATUS AND PROCEDURES

BY CHARLES SHEARD AND LOUIS A. BRUNSTING

*(From the Division of Physics and Biophysical Research and the Section on Dermatology and Syphilology, The Mayo Foundation Rochester)*

(Received for publication April 29, 1929)

The color of the skin, to a certain extent, serves as an index of the state of well being, or the converse, and has been interpreted, after various modes and manners of expression, as evidence of health or disease. On the one hand there is the tanned, full-blooded skin with the texture and sheen of velvet and, on the other hand, the pale, anemic, wrinkled and lifeless skin. To be sure, the normal person exhibits a wide variation or range in the color attributes of the skin, depending on the race, habitude, occupation and exposure to the elements. Without doubt variations in environment or bodily temperature and psychic emotion or unrest play a part in the composite of factors which go to make up the color of the skin. In pathologic conditions, in which there may be abnormalities or derangements of pigmentation and of the vascular distribution (both as to quantity and quality of blood) there is a wide range of variations in the color of the skin. Some of these changes may be recognized readily by the eye, others cannot be so recognized and, if appreciated, cannot be given quantitative estimation by the clinical methods in vogue.

It would seem as though greater accuracy should be introduced in clinical records regarding the color of the skin. This is particularly true in such conditions as jaundice, hemochromatosis, anemia, polycythemia, cyanosis, Addison's disease and in other pigmentary and vascular disturbances in which color plays a part in the diagnosis, and influences judgment regarding favorable or unfavorable progress during the course of treatment.

That there has not been uniformity in correctly or adequately expressing the color of the skin is attested by numerous clinical reports

in the literature in which are used such expressions as reddish hue, bluish hue, death-like, bronzy, livid, cyanotic, and the like. The language used for expressing conceptions regarding color has been rather figurative or from an artist's viewpoint. This has been due to the lack of standards, and chiefly for the reason that color has not been expressed in terms of its three attributes: relative luminosity (brilliance), dominant wavelength (hue) and purity (saturation).

#### TINTOMETRIC METHODS

Various tintometric or colorimetric methods of estimating color have been described in the literature (2, 3, 9, 13). Various color plaques are employed in such tests and are in character analogous to the Tallquist scale for the estimation of hemoglobin. Such tintometric and colorimetric methods of estimating and recording color are doubtless of value to clinical medicine, especially when subjected to some definite system of color analysis, such as the Munsell disk or the Bradley top. However useful such methods may be, they are open to the objection that they do not in any wise analyze the spectral light reflected by the skin and therefore cannot record in terms of the three fundamental attributes of color. Furthermore, pigments and spectral colors are vastly different. The spectral colors of white light, such as sunlight, are pure colors, can be measured as to intensity, purity, and so on, and are, therefore, readily standardized. For instance, spectral yellow of a given wavelength (for example, 590 millimicrons or 5900 Angstrom units) is always the same yellow and can be reproduced exactly.

#### ATTRIBUTES OF COLOR

White light can be made spectrally from certain quantities of red, green and blue of limited wavelength values or, again, from certain quantities of orange, green and blue of limited wavelength values according to the tables of Maxwell (7) and others (10). Unless a color is described according to its attributes, nothing definite is stated regarding the color. The three attributes of color are relative luminosity, dominant wavelength and purity. Relative luminosity or brilliance is necessary if color exists at all. It is often spoken of as brightness, tint, value, or visual brightness. In this particular attribute,

colors may be classified as equivalent to some member of a series of grays of which black and white are the extremes. Relative luminosity simply tells how much of a standard source of light (such as sunlight) the given color is capable of reflecting. By dominant wavelength or hue is meant that attribute of the color which permits it to be classed as reddish, yellowish, greenish or bluish. The hue or tone depends on the wavelength only. Percentage of purity or saturation determines the degree of hue, stating how vivid or distinct it is. Thus the percentage of purity defines how red or how yellow, and so forth, a color is. For a full discussion of these attributes of color and allied topics, reference should be made to the report of the Committee on Colorimetry of the Optical Society of America (14).

Since the pigmentation of the skin is largely due to melanin (at least in brunets, negroes and those suffering from Addison's disease), the degree of pigmentation, which affects the brilliance and purity only, may be thought of roughly as a smoke screen (in some persons very slight and in others very dense) laid down between solar radiation, on the one side, and the blood in the peripheral capillaries on the other side of the dividing medium, the epidermis. In other words, the presence of a pigment, such as melanin, will not affect the dominant wavelength but will influence the relative luminosity and saturation. On the other hand, the presence of abnormally large quantities of blood at the periphery of the body may influence the dominant wavelength and perchance also the other attributes of the color of the skin. The effects of pigmentation and superficial blood are the subjects of the second and third papers of this series dealing with the color of the skin.

It is readily seen, therefore, in a consideration of the color of the skin (which is a semi-opaque body) that systems dependent on color matching with the human eye fail to analyze sufficiently the light reflected from the skin. It is physically impossible for the human eye to estimate color quantitatively and to analyze it according to its attributes. Rowntree and Brown (9) developed a universal skin tintometer, adaptable to clinical medicine, which consists of nine separate color scales. Matchings were made and grades given to various selected areas of skin under conditions least likely to introduce error. These readings were then subjected to the Munsell (8) system of color measurement.

in the literature in which are used such expressions as reddish hue, bluish hue, death-like, bronzy, livid, cyanotic, and the like. The language used for expressing conceptions regarding color has been rather figurative or from an artist's viewpoint. This has been due to the lack of standards, and chiefly for the reason that color has not been expressed in terms of its three attributes: relative luminosity (brilliance), dominant wavelength (hue) and purity (saturation).

#### TINTOMETRIC METHODS

Various tintometric or colorimetric methods of estimating color have been described in the literature (2, 3, 9, 13). Various color plaques are employed in such tests and are in character analogous to the Tallquist scale for the estimation of hemoglobin. Such tintometric and colorimetric methods of estimating and recording color are doubtless of value to clinical medicine, especially when subjected to some definite system of color analysis, such as the Munsell disk or the Bradley top. However useful such methods may be, they are open to the objection that they do not in any wise analyze the spectral light reflected by the skin and therefore cannot record in terms of the three fundamental attributes of color. Furthermore, pigments and spectral colors are vastly different. The spectral colors of white light, such as sunlight, are pure colors, can be measured as to intensity, purity, and so on, and are, therefore, readily standardized. For instance, spectral yellow of a given wavelength (for example, 590 millimicrons or 5900 Angstrom units) is always the same yellow and can be reproduced exactly.

#### ATTRIBUTES OF COLOR

White light can be made spectrally from certain quantities of red, green and blue of limited wavelength values or, again, from certain quantities of orange, green and blue of limited wavelength values according to the tables of Maxwell (7) and others (10). Unless a color is described according to its attributes, nothing definite is stated regarding the color. The three attributes of color are relative luminosity, dominant wavelength and purity. Relative luminosity or brilliance is necessary if color exists at all. It is often spoken of as brightness, tint, value, or visual brightness. In this particular attribute,

briefly the essential points of construction and operation. A spherical lamp house lined with zinc-oxide paint and holding two 400 watt stereopticon bulbs serves as a source of white light which remains

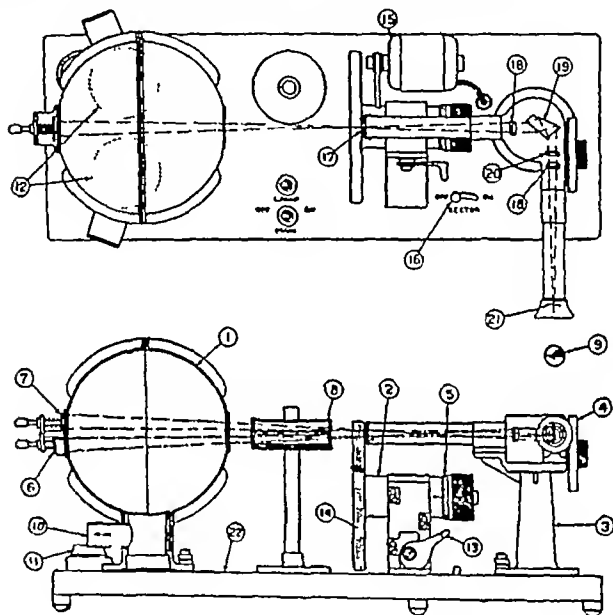


FIG. 2 CROSS-SECTIONAL DIAGRAMS SHOWING THE WORKING PARTS OF THE SPECTROPHOTOMETER

1, spherical light house, 2, photometer, 3, spectrometer, 4, wavelength scale, 5, photometric scale, 6, holder for standard sample, 7, holder for reflection samples, 9, field of view through the eye slit, 14, sector disks, 17, entrance slit, 19, dispersion prism, and 20, biprism

constant during all the readings. Smooth magnesium blocks at the rear of the sphere serve to reflect the light through various transparent mediums (liquids) which may be placed in the path in the support (fig. 2). In such experiments as these, in which an opaque object

such as the skin is to be studied, the holder for liquids is not used. One of the magnesium blocks is replaced by the area of skin to be investigated. We have recently devised a substitute for the regular attachment to the spectrophotometer which is water-cooled and which enables any portion of the body to be examined. This device will be described in subsequent paragraphs. The two beams of light, reflected from the magnesium block and the skin, respectively, enter the spectrophotometer to be analyzed. The spectrometer is essentially the ordinary constant deviation type of instrument except for the addition of a biprism and an observing slit at the exit. The spectrometer is set at any desired wavelength by rotating the wheel, 4, which serves to turn the prism of the spectrometer. The amount of light admitted to match brightness or saturation value is determined by the variation in the sector openings in the rotating disks in the photometer. This is controlled by a calibrated drumhead, 5. The image at the observing exit slit consists of two semicircular colored fields separated by a horizontal line. The hue of these areas will be the same and will depend on wavelength only. Equality of brightness can be obtained by turning the photometer drumhead until an exact match is obtained. This reading (0 to 100 per cent) is then recorded as the percentage of light reflected (or transmitted) at the particular wavelength at which the spectrometer is set. Throughout this particular series of observations the entrance slit was adjusted to give constant reflection of light from the source of white light throughout the whole spectrum, and the entrance and exit slits were thereafter maintained at fixed values.

Readings are taken of the specimen under consideration by turning the calibrated wheel, 4, over a space of 10 to 20 millimicrons. The percentage of light reflected at each wavelength is read directly from the matching of brilliance obtained by turning the drumhead, 5, at the same time. A spectrophotometric curve can then be plotted, using wavelengths of light as the abscissas and the percentages of reflection as the ordinates. This gives graphic data which are readily transferable into other values, as will be shown. From such a spectrophotometric curve an experienced observer can determine certain characteristics of the object under consideration by studying the form of the reflection curve in particular regions of the spectrum. Such

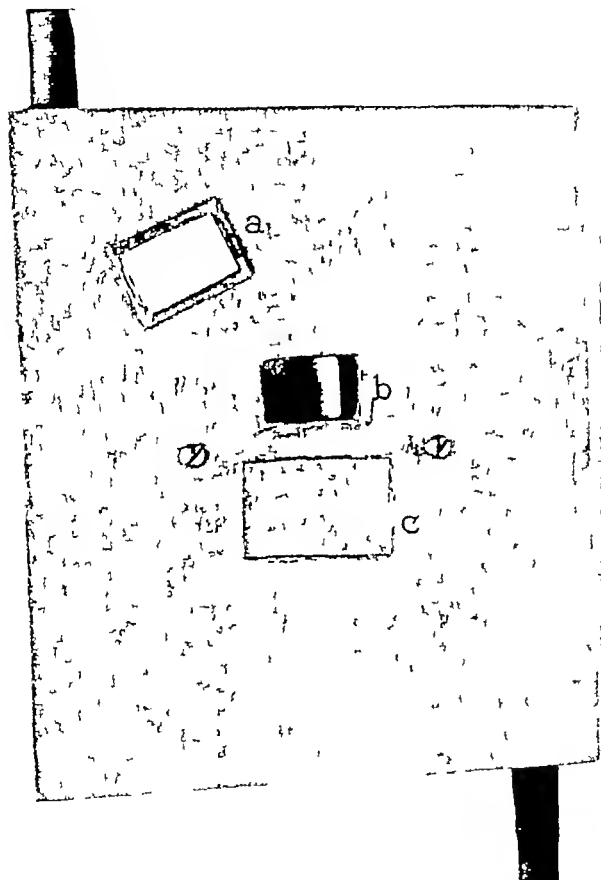


FIG 3 WATER CELL ATTACHMENT TO REPLACE USUAL HOLDERS FOR STANDARD AND SUBSTANCE TO BE EXAMINED BY METHODS OF REFLECTION

*a* brass plaque coated with magnesium carbonate *b*, aperture in the water cooled holder which is attached to the instrument, and *c*, standard in position

spectrophotometric readings, since they measure the spectral distribution of the color of the object examined and so actually measure the light stimuli, are independent of the condition of color vision of the observer's eye. Therefore, all observers should get the same results. Also, when a colored object is viewed by the same observer with the naked eye under lights of different quality, the sensation produced under each light will be different although the color itself has not changed physically. Measurements made with a spectrophotometer

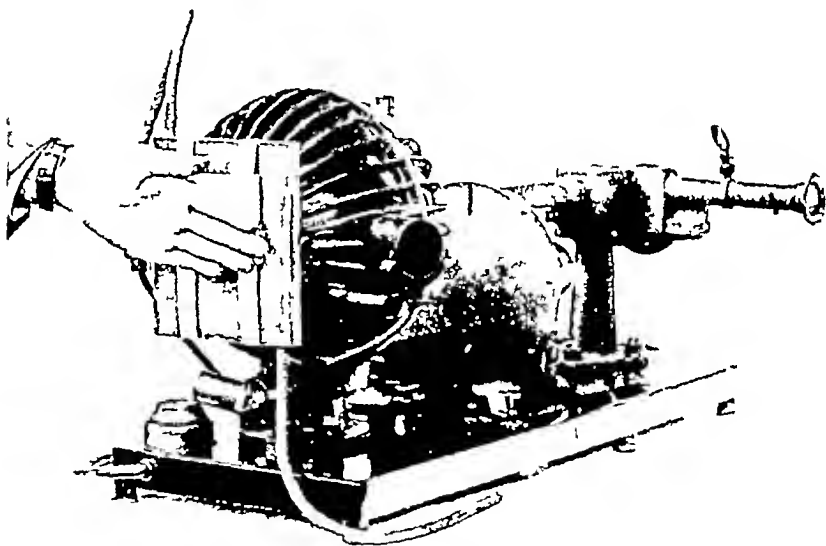


FIG 4 KEUFFEL AND ESSER COLOR ANALYZER WITH WATER CELL MODIFICATION. ATTACHED, THE RELATION OF THE INSTRUMENT TO SAMPLE TO BE STUDIED IS SHOWN

are independent of the quality of the source of light as contrasted to color observed by the method of colorimetry or direct matching

#### AUTHORS' WATER-COOLED DEVICE TO PERMIT SPECTROPHOTOMETRIC EXAMINATIONS OF LIVING MATERIALS

The Keuffel and Esser color analyzer was originally adapted to record reflection values from such opaque flat objects as paper, cloth, leather, soap, flour, paints, and the like, and to serve as a basis of color

specification and color control. To adapt the apparatus so that areas of human skin could be measured, it was found necessary to modify the specimen holder. Such easily available portions of the body as the back of the hand or the fingers could be read with the original apparatus, although it was necessary to allow time for the cooling of the lamp-housing in spite of the fact that a current of air was allowed to play between the lamps and the specimen being examined. In devising a practical specimen holder it was borne in mind that although the distance from the source of light could be increased to eliminate to some extent the effects of heat, the illumination suffered in direct proportion. A solution to the problem was found in a thin metal water cell (figs 3 and 4) so constructed as to fit in the place of the ordinary holders and be directly attached to the spherical lamp-housing. The circulation of the water serves to maintain the cell at a constant cool temperature. Small rectangular perforations are cut into the face of the cell, one above the other. The distal surfaces are covered, in the lower case by a white reflecting surface to take the place of the magnesium block in the original standard. The sample to be tested is brought flush against the upper opening, separated from the metal only by a thin layer of felt which is conveniently changed for purposes of cleanliness and which acts to prevent a change of temperature in the skin. A small, removable plaque, faced on the one side with white magnesium carbonate paint, was constructed and placed in the aperture ordinarily used for reflection purposes in case the instrument was to be used for transmission spectral studies. It is necessary to take the same precaution with the white facings on these metal plaques as with the standard magnesium blocks, for they must be kept smooth and clean throughout. They can be checked at the beginning of any particular experiment to determine whether the reading of brilliance on the sector scale is approximately 100 per cent.

#### CONVERSION OF SPECTROPHOTOMETRIC DATA INTO TERMS OF COLOR EXCITATION VALUES

In order to convert the curves of spectral reflection (or transmission) into a form which can be interpreted directly in terms of color excitation values, it is necessary to make certain computations. Probably

the most fundamental of all psychophysical data relating to color are the three color excitation curves which represent graphically the laws of a three-color mixture. The principal data on these relationships we owe to Maxwell (7), Abney (1), and König and Dieterici (6). The results of the latest investigations on this matter, reduced to an equal energy spectrum and referred to average noon sunlight, are plotted in figure 5.

Spectrophotometric data are given generally in the form of curves of spectral transmission or reflection. Such curves require combina-

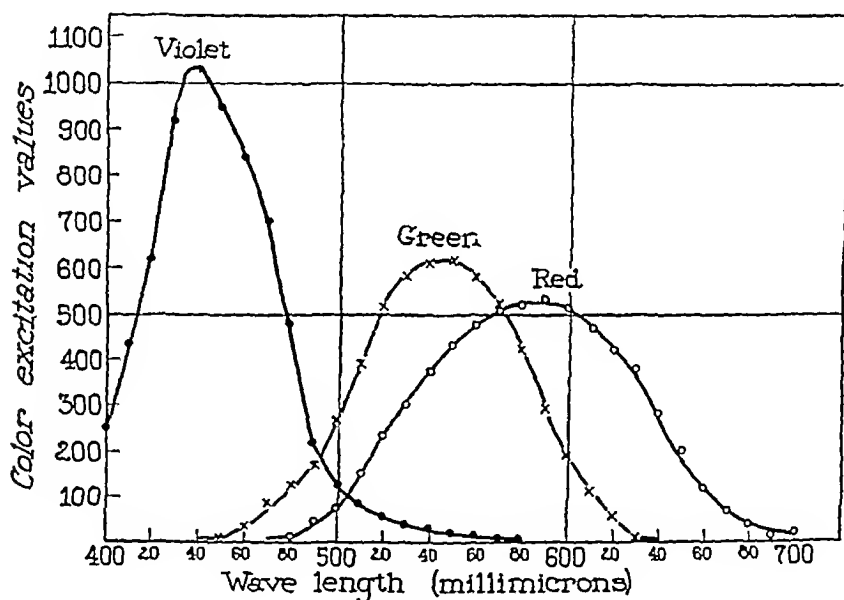


FIG 5 ELEMENTARY COLOR EXCITATIONS FOR DIFFERENT WAVELENGTHS

tion with a certain energy distribution representative of the particular source with which the object is illuminated in order that they shall become determinative of a definite color. The process of reducing any given set of spectrophotometric data to excitation color values is therefore as follows: (1) multiply each of the ordinates of the transmission or reflection curve by the corresponding ordinate of the energy distribution curve of the source, (2) multiply each of the ordinates of the resulting curve by the corresponding ordinate of each of the color excitation functions as shown in figure 5, this being a separate opera-

tion for each of the three excitations and yielding three separate curves which represent the respective excitation values for each wavelength of the given stimulus, (3) determine separately the areas of the three curves thus found, and (4) reduce the three areal values thus obtained to percentage form, so that their determined ratio re-

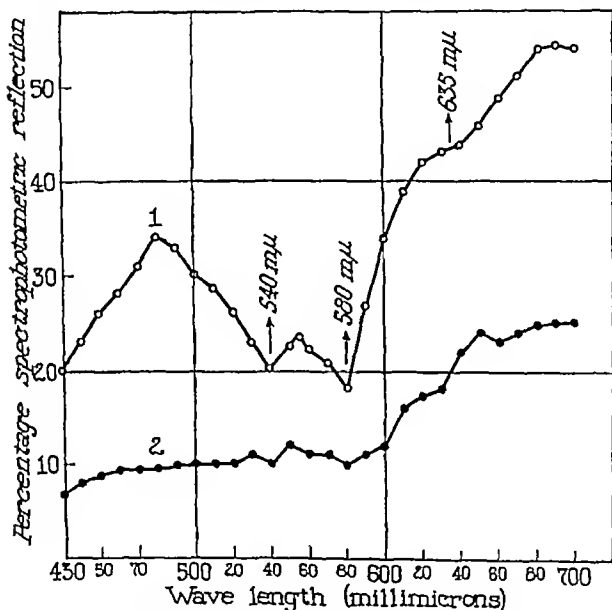


FIG. 6 SPECTROPHOTOMETRIC CURVES OBTAINED BY REFLECTION FROM THE FINGERS

Curve 1, normal blond, curve 2, normal mulatto

mains unchanged but their sum becomes equal to 100. The color excitation values can then be expressed by means of two numbers, representing the red and violet excitation percentages, that for the green being obtainable by subtracting the sum of these two values from

100 Complete details on these points are given in the report of the Committee on Colorimetry of the Optical Society of America (14)

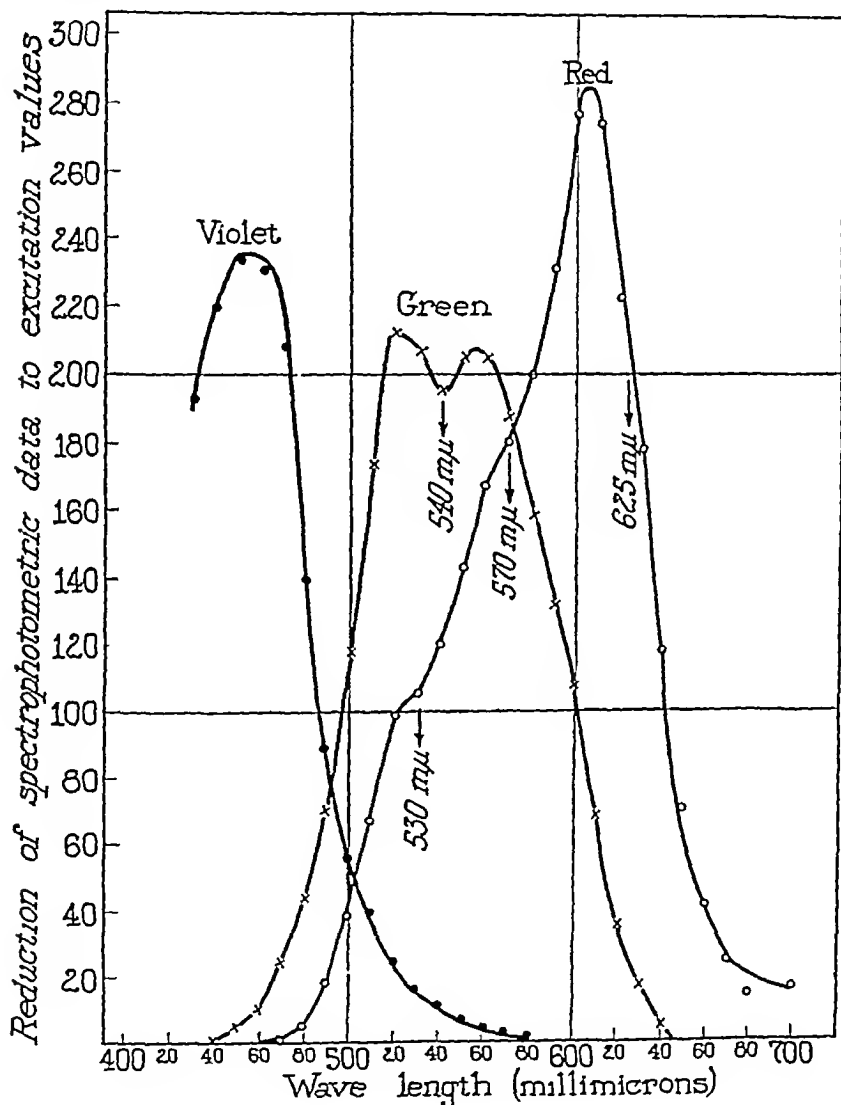


FIG 7 ANALYSIS OF THE SPECTROPHOTOMETRIC DATA (SHOWN GRAPHICALLY IN FIGURE 6) INTO THE THREE PRIMARY EXCITATION CURVES

Figure 7 contains the red, green and violet excitation curves for the spectrophotometric data given in curve 1 of figure 6, which was

obtained in the case of a young normal blond This curve of spectrophotometric reflection, as well as that which gives the subsequent analysis of this reflection curve into fundamental red, green and violet

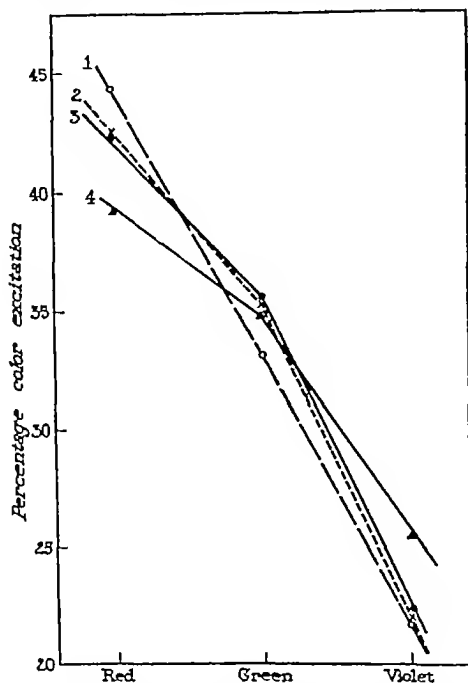


FIG 8 PERCENTAGE COLOR EXCITATION VALUES

Curve 1, normal brunet, curve 2, a case of Addison's disease, curve 3, a normal negro, and curve 4, a case of scleroderma All data were obtained from fingers held slightly below heart level

color values, shows the presence of spectral bands at 625, 580 and 540 millimicrons respectively The bands at 580 and 540 millimicrons are due to the absorption of light by the blood (oxyhemoglobin bands),



fundamental colors, red, yellow and violet, respectively, proceeding from 700 to 430 millimicrons (for example, the separate excitation color curves shown in figure 7) gives the total red, green and violet excitation values respectively. From the sum of the three separate summation values it is easily possible to obtain the percentage of red, green and violet color excitations, respectively. Figure 8 gives a group of such percentage color excitation values in the case of a normal brunet (curve 1), a case of Addison's disease (curve 2), a normal negro (curve 3) and a case of scleroderma (curve 4). In the subsequent papers which deal with the rôle of pigmentation and of superficial blood a number of such analyses will be presented and discussed.

In addition to the data regarding the total color excitation values and the relative percentages of red, green and violet, respectively, in the white light reflected by the skin, the dominant wavelength or hue and the percentage purity or saturation can be determined by the use of charts provided for this purpose. A reproduction of such a chart which may be obtained in large dimensions from Keuffel and Esser, is shown in figure 9. Using this color triangle and taking the percentages of red and violet determined from an analysis of any spectrophotometric curve into its fundamental red, green and violet components, we are able to find the dominant wavelengths (or hues) as well as the degrees of purity and relative luminosities.

#### SUMMARY

In this paper we have presented and briefly commented on (1) tintometric methods for estimating the color of the skin, (2) the fundamental attributes of color, (3) spectrophotometric apparatus and methods of procedure for obtaining curves of the reflection, by the skin, of light of various wavelengths, (4) the authors' water-cooled device to enable spectrophotometric determinations to be made on living materials, (5) spectrophotometric analyses of the color of the skin, and (6) conversion of spectrophotometric data into terms of color excitation values, percentages of fundamental red, green and violet reflected by the skin, dominant wavelength, purity and relative luminosity.

## BIBLIOGRAPHY

- 1 Abney, W de W , *Researches in Colour Vision and the Trichromatic Theory*  
London, Longmans, Green and Co , 1913, 431 pp
- 2 Freund, Leopold, and Novak, Franz, *Wien Mun Wchnschr* , 1925, xxxviii,  
760 Neuere Behelfe zur Messung von Hautrötungen und-braunungen
- 3 Freund, Leopold, and Valenta, Eduard, *Deutsch med Wchnschr* , 1922,  
xlviii, 839 Eine Rötungsskala
- 4 Gibson, K S , *Glazebrook's Dictionary of Applied Physics*, Macmullan Co ,  
1923, 737 Spectrophotometry
- 5 Keuffel, Carl W , *J Optic Soc Am and Rev Sci Instr* , 1925, vi 403 A  
Direct Reading Spectrophotometer
- 6 König, A , and Dieterici, C , *Ztschr f Psychol und Physiol d Sinnesorg* ,  
1892, iv, 241 Die Grundempfindungen in normalen und anomalen Farben-  
systemen und ihre Intensitätsverteilung im Spektrum
- 7 Maxwell, J C , *Scientific papers*, 1, 410 On the Theory of Compound Colors  
and the Relations of the Colors of the Spectrum
- 8 Munsell, A , *A Color Notation* Baltimore, Munsell Color Co , 6th Ed ,  
1923
- 9 Rowntree, L G , and Brown, G E , *Am J Med Sci* , 1925, clxx, 341 A  
Tintometer for the Analysis of the Color of the Skin
- 10 Sheard, Charles, *Physiological Optics* Chicago, Cleveland Press, 1918, 513  
pp
- 11 Sheard, Charles, *Science*, 1926, lxiv, 70 Analysis of the Color of the Skin  
and Its Significance
- 12 Sheard, Charles, and Brown, G E , *Arch Int Med* , 1926, xxxviii, 816  
The Spectrophotometric Analysis of the Color of the Skin and the Observa-  
tions by This Method in Normal and in Pathologic Subjects
- 13 Todd, T W , and Van Gorder, L , *Am J Phys Anthropol* , 1921, iv, 239  
The Quantitative Determination of Black Pigmentation in the Skin of the  
American Negro
- 14 Troland, L T , *J Optic Soc Am and Rev Sci Instr* , 1922, vi 527  
Report of Committee on Colorimetry.

# THE COLOR OF THE SKIN AS ANALYZED BY SPECTROPHOTOMETRIC METHODS

## II THE RÔLE OF PIGMENTATION<sup>1</sup>

BY LOUIS A. BRUNSTING AND CHARLES SHEARD

*(From the Section on Dermatology and Syphilology and the Division of Physics and Biophysical Research The Mayo Foundation, Rochester)*

(Received for publication April 29, 1929)

The color of the skin of human beings depends chiefly on the presence of pigment and superficial blood. Spectrophotometric data on the reflection of visible light from the surface of the skin were obtained in a number of subjects possessing various degrees of pigmentation. Analysis of the spectrophotometric curves obtained for normal and pathologic subjects into terms of red, green and violet excitation color values, and subsequent conversion of these values into expressions of dominant wavelength, purity and relative luminosity, indicate certain fairly constant relations of content of pigment to the color of the skin.

Certain secondary factors contributing to the amount of light reflected from the skin, such as surface dirt, oil and moisture, were eliminated by making reasonably sure that the area under investigation was clean. Preliminary readings apparently showed that the presence of a small amount of hair or the obliteration of the normal rugae of the surface of the skin did not affect the percentage of light reflected to any considerable extent. Only under pathologic conditions, involving especially the presence of an abundance of scales, were the reflection values materially altered. It may be said in general that the local contour may be disregarded as a factor in affecting the color of the skin.

<sup>1</sup> The material in this paper was submitted by Dr. Brunsting to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Dermatology and Syphilology, 1929. Work done in the Division of Physics and Biophysical Research.

Schultze (4) attempted an analysis of the utilization of light by the skin as manifested by its spectral reflection and transmission. He used a photographic-photometric method of recording the light reflected by the skin, further analyzed the results by means of a Marten's polarization photometer, and plotted the curves in percentages of reflected light. The results of Schultze give information regarding the total reflection of visible light by the skin in various areas of the body under controlled conditions. Although the results do not embody a monochromatic analysis of the reflected light, they show that pigmentation tends to diminish the amount of light reflected by the skin.

Dorno (2), using similar methods, observed variations in the reflection of light by the skin as affected by race, age and sex. He likewise noted general diminution of the light reflected under conditions of increased pigmentation.

Sonne (8) estimated that the skin of a normal person was capable of reflecting approximately 35 per cent of the visible light falling on it.

Sheard (5), and Sheard and Brown (6) were among the first to apply spectrophotometric methods to clinical medicine in a series of observations on the color of the skin in various normal and pathologic conditions, and were probably the first to analyze the resultant spectrophotometric reflection curves into percentages of fundamental red, green and violet excitation color values, dominant wavelength and saturation. The series of investigations reported here is essentially a continuation of their work.

The direct-reading spectrophotometer, known as the Keuffel (3) and Esser color analyzer, with the author's water-cell modification (1), was used throughout these investigations. The cleaned surface of skin was brought flush against one aperture of the water-cooled cell, with constant conditions of temperature and pressure. Readings were made directly in terms of the percentage of light reflected from the surface.

In order to evaluate the rôle of pigment as a factor in contributing color to the skin, comparative records were obtained on subjects presenting varying gradations of pigmentation under normal and abnormal conditions. The following distinctions were observed: (1) variations over several areas of the body of the same subject, (2)

variations among different subjects classified according to type, as blond or brunet, (3) variations in subjects of different races, including the so-called white, black, yellow and red races, (4) a comparison of normal values with the data obtained for subjects manifesting abnormal degrees of pigmentation, such as in Addison's disease, arsenism, argyria and jaundice

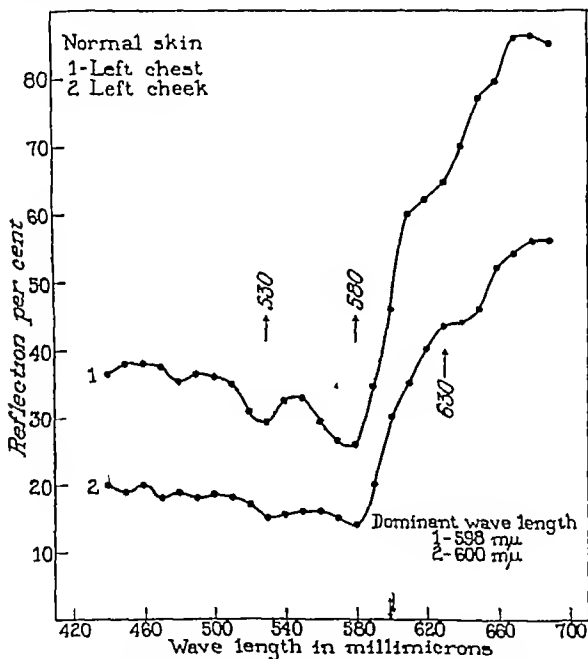


FIG 1 SPECTROPHOTOMETRIC REFLECTION CURVES OF THE SKIN OF A NORMAL SUBJECT 1, CHEST, 2, CHEEK

#### VARIATION IN PIGMENTATION IN THE SAME SUBJECT

It is self-evident that the normal skin presents an amount of pigment roughly proportional to the amount of habitual exposure to the

sun and the elements. There is a wide variation in pigmentary response in the same subject, as well as in different subjects, to exposure to sunlight.

Figure 1 represents two curves from the same subject showing a record of the light reflected from the skin of the cheek as compared with the chest. In general form the curves are similar, that is, they maintain a similar linear relationship. However, there is a constant difference in the percentage of light reflected throughout the visible spectrum, more being reflected from the chest (which is ordinarily protected from sunlight, as in this instance) than from the cheek, which is more or less exposed. This difference in the amount of reflection represents a distinction in relative luminosity or brilliance of the areas under consideration. The curves show decreased percent-

TABLE 1  
*Analysis of color values obtained from spectrophotometric curves of figure 1*

| Curve | Area  | Total color<br>excitation | Red             | Green           | Violet          | Dominant<br>wave-<br>length | Purity          | Relative<br>luminosity |
|-------|-------|---------------------------|-----------------|-----------------|-----------------|-----------------------------|-----------------|------------------------|
|       |       | <i>units</i>              | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>milli<br/>microns</i>    | <i>per cent</i> | <i>per cent</i>        |
| 1     | Chest | 6,708                     | 41.5            | 32.7            | 25.8            | 598                         | 26              | 36.7                   |
| 2     | Cheek | 3,703                     | 44.1            | 31.7            | 24.2            | 600                         | 33              | 20.5                   |

ages of reflection at the regions corresponding, approximately, to 540, 580 and 630 millimicrons, respectively. That these are related to the presence of superficial blood seems likely, as stated by Sheard and Brown (6), absorption at 540 and 580 millimicrons is due to oxyhemoglobin and at 630 millimicrons is probably due to hematin. Zones of decreased reflection, as shown in the curves, are more pronounced in the reflection curve obtained from the surface of the chest than from the cheek, inasmuch as the amount of pigment interposed is correspondingly less. The influence of pigment on these absorption bands is to minimize them. This will be brought out more fully later in the paper.

Table 1 represents a summary of data obtained by an analysis of the spectrophotometric curves in figure 1, following the methods outlined by the Committee on Colorimetry of the Optical Society of

America (10), these methods consist of the translation of reflection values into terms of color, namely hue or dominant wavelength, purity, and brilliance or relative luminosity. In general, the quantity and quality of color are expressed in terms of stimulus or color excitation units, which include the summated values of the red, green and violet. These values determine the index of saturation or purity of hue as well as the dominant wavelength. The exact procedure used in the analyses is discussed elsewhere (1, 7).

Pigmented skin reflects less light than nonpigmented skin, and stimulates fewer color excitation units, giving a diminished percentage of total brilliance. The skin of the cheek, in this instance, has a relative luminosity value of only 20.5 per cent, as compared to 36.7 per cent for the skin of the chest. The relative percentages of red, green and violet are an index to the hue and the degree of hue, saturation or purity. In this instance there appear to be higher percentages of red and lower percentages of green and violet color excitation values obtained from the cheek than from the chest and correspondingly different percentages of purity. Nevertheless, the red, green and violet values in each instance maintain a linear relationship in spite of the difference in color excitation units produced by the two areas under consideration. This holds true regardless of changes in the quantity of pigment present.

#### VARIATIONS AMONG DIFFERENT SUBJECTS CLASSIFIED ACCORDING TO TYPE AS BLOND OR BRUNET

A series of blonds and brunets, selected at random, was investigated with reference to variations of pigment content of the skin of widely separated areas of the body. Spectrophotometric reflection curves were obtained from the cheek, chest, inner side of the arm and the dorsum of the middle finger, the hand in the last instance being held about 18 cm. above heart level to reduce the quantity of the superficial venous blood. These curves were analyzed in each instance into the attributes of color, and the resultant data are given in table 2.

Quantitative estimates of the intensity of pigmentation can be made readily from these data. The individual values for the amounts of red, green and violet are maintained at a fairly constant level. This results in little, if any, change in the hue or dominant wavelength,

which remains approximately at 590 millimicrons. This corresponds to pure spectral yellow. Likewise the purity or saturation is a func-

TABLE 2

*Analyses from spectrophotometric curves of normal blonds and brunets to demonstrate variation of pigment over body areas more or less exposed to light*

| Case | Type    | Age | Sex | Area                   | Total excitation | Red      | Green    | Violet   | Hue           | Purity   | Relative luminosity |
|------|---------|-----|-----|------------------------|------------------|----------|----------|----------|---------------|----------|---------------------|
|      |         |     |     |                        | units            | per cent | per cent | per cent | milli-microns | per cent | per cent            |
| 1    | Blond   | 28  | F   | Chest                  | 7,295            | 41.0     | 33.0     | 26.0     | 595           | 27       | 39.0                |
|      |         |     |     | Hand above heart level | 6,812            | 40.1     | 32.3     | 27.6     | 600           | 21       | 36.3                |
| 2    | Blond   | 22  | F   | Chest                  | 8,202            | 39.2     | 34.3     | 26.5     | 587           | 26       | 44.3                |
|      |         |     |     | Hand above heart level | 6,775            | 39.3     | 34.4     | 26.3     | 587           | 26       | 36.9                |
| 3    | Blond   | 18  | F   | Chest                  | 8,422            | 39.5     | 34.2     | 26.3     | 590           | 27       | 46.1                |
|      |         |     |     | Hand above heart level | 7,848            | 40.0     | 33.8     | 26.2     | 590           | 27       | 42.7                |
| 4    | Blond   | 32  | M   | Chest                  | 8,579            | 39.0     | 34.1     | 26.9     | 590           | 24       | 44.2                |
|      |         |     |     | Cheek                  | 5,973            | 40.3     | 32.8     | 26.9     | 598           | 23       | 33.0                |
| 5    | Brunet  | 35  | M   | Inner side of arm      | 6,924            | 43.9     | 34.6     | 21.5     | 590           | 43       | 41.7                |
|      |         |     |     | Cheek                  | 4,372            | 41.2     | 34.0     | 24.8     | 590           | 31       | 24.3                |
| 6    | Brunet  | 36  | M   | Inner side of arm      | 6,601            | 41.3     | 35.2     | 23.5     | 587           | 37       | 37.3                |
|      |         |     |     | Cheek                  | 3,710            | 43.9     | 33.3     | 22.8     | 597           | 37       | 20.9                |
| 7    | Brunet* | 32  | F   | Chest                  | 5,024            | 42.0     | 32.6     | 25.2     | 596           | 30       | 28.1                |
|      |         |     |     | Hand above heart level | 5,706            | 41.3     | 34.0     | 24.7     | 592           | 28       | 32.4                |
|      |         |     |     | Cheek                  | 4,904            | 41.2     | 32.8     | 26.0     | 596           | 27       | 26.8                |

\* Much generalized tanning

tion of the amount of red, green and violet color excited. The blond types manifested a more constant percentage of purity, ranging from 21 to 37 per cent, than did the brunet types, whose percentage varied

from 27 to 37 per cent, with one value as high as 43 per cent. In general, as the percentage of red summation increased, the percentage of violet decreased, with very little variation in the percentage of green color. That is to say, the linear relationships were preserved in these types of light and dark complexions.

The brilliance, however, underwent marked variation, and apparently in direct proportion to the absence of pigment present in the specimen of skin under observation. The amount of pigment de-

TABLE 3  
*Analyses from spectrophotometric curves of a Negro, Chinese and Japanese*

| Type     | Age | Sex | Area                   | Total excitation | Red      | Green    | Violet   | Blue          | Purity   | Relative luminosity |
|----------|-----|-----|------------------------|------------------|----------|----------|----------|---------------|----------|---------------------|
|          |     |     |                        | units            | per cent | per cent | per cent | milli microns | per cent | per cent            |
| Negro    | 42  | M   | Chest                  | 3,316            | 42.4     | 33.8     | 23.8     | 590           | 35       | 18.6                |
|          |     |     | Hand above heart level | 1,272            | 44.3     | 36.3     | 19.4     | 585           | 50       | 7.5                 |
| Chinese  | 40  | M   | Chest                  | 6,329            | 39.8     | 35.9     | 24.5     | 585           | 33       | 33.7                |
|          |     |     | Malar region           | 5,753            | 42.9     | 34.0     | 23.1     | 590           | 33       | 32.0                |
|          |     |     | Hand above heart level | 4,863            | 39.0     | 34.7     | 26.3     | 585           | 28       | 27.2                |
| Japanese | 30  | M   | Inner side of arm      | 5,240            | 39.5     | 33.0     | 27.5     | 595           | 22       | 28.0                |
|          |     |     | Hand above heart level | 4,409            | 40.4     | 34.8     | 24.8     | 587           | 31       | 24.3                |

termines the total amount of the summated excitation values for red, green and violet. For instance, in the case of a brunet, skin from an area such as the chest or the inner side of the arm (which had received very little exposure to sunlight) would reflect in some cases more light than skin (blond) which had become heavily pigmented through constant exposure. Although pigment affects the brilliance of color, in and of itself, it does not alter the values of the dominant wavelength and purity. Blonds and brunets are undistinguishable except for relative variation in brilliance or luminosity.

*Variations in subjects of different races*

The color of the skin has always held an important place among typical criteria of race. Records have been obtained on a number of subjects (fig 2), of which the values for a full-blooded negro, a Chinese and a Japanese are tabulated for purposes of comparison (table 3)

The skin of the negro reflects considerably less light than the skin of the Japanese or the Chinese. A percentage of relative luminosity

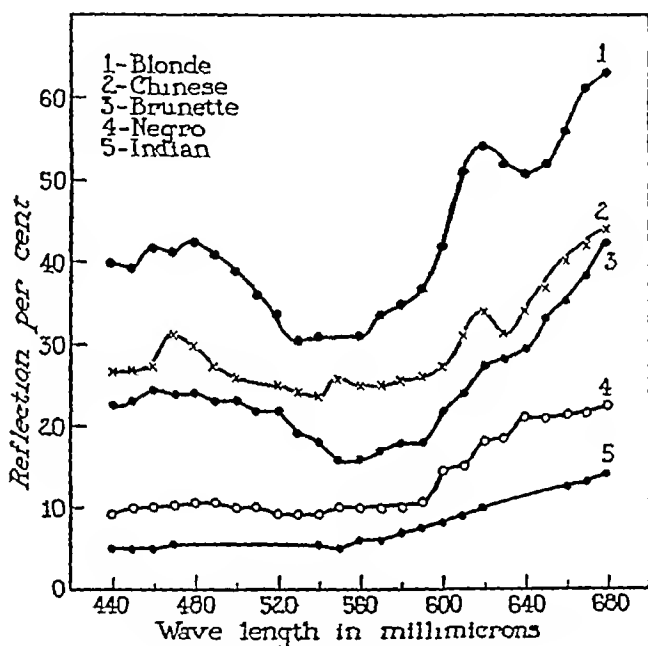


FIG 2 A SERIES OF SPECTROPHOTOMETRIC CURVES BY THE REFLECTION METHOD OBTAINED FROM THE SKIN OF SUBJECTS OF VARIOUS RACES

or brilliance of 7.5 was obtained in the area of skin from the dorsum of the hand of the negro. The surface of the chest, however, which was ordinarily covered, reflected 18.6 per cent of the light falling on it. Moreover, the percentage values for red, green and violet appear to be in a different relation to each other. Nevertheless they have maintained the same linear relationship, as demonstrated in the skin of the white subject. The purity of the color in the area of the hand reaches

50 per cent, but the hue or dominant wavelength remains constantly near the region of 590 millimicrons

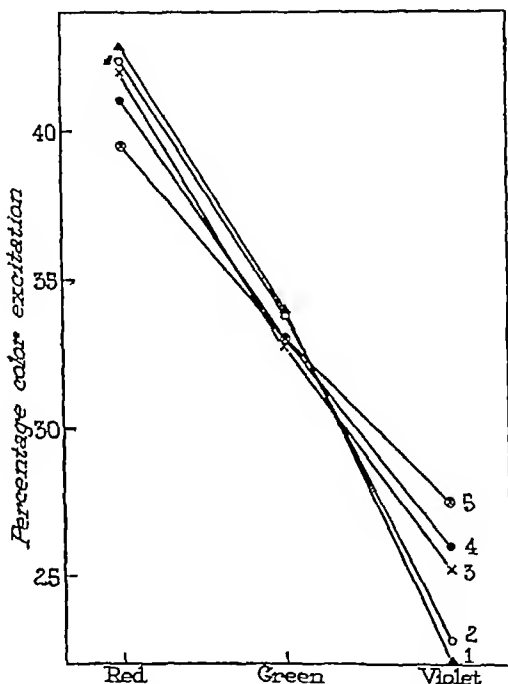


FIG 3 PERCENTAGE COLOR EXCITATION VALUES OBTAINED FROM SPECTROPHOTOMETRIC DATA IN PERSONS EXHIBITING VARYING DEGREES OF PIGMENTATION 1, CHINESE, 2, NEGRO, 3, BLOND, 4, BRUNET AND 5, JAPANESE

Data obtained by the reflection method, after being analyzed into fundamental red, green and violet components for the blond, brunet, negro, Chinese, and Japanese have been plotted and are shown graphically in figure 3. On the basis of specification of color alone,

a definite distinction cannot be made as to whether a subject is a member of one race or another

Todd and Van Gorder (9) in anthropologic studies on the skin of the American negro, utilized the system of spinning tops (Bradley) to estimate the composite coloring of the skin with particular regard to the content of the black pigment. This work was part of a larger research on the correlation of various physical features and was presented as an effort to promote greater accuracy in the quantitative estimation of the color of the skin. The method does not provide for the analysis of color as defined according to its attributes of hue, purity and relative luminosity, but tabulates the records obtained in the registration of brightness, or lack of it, in the skin of the negro. Our work confirms the impression of these authors that the color of the skin is a faulty racial test when used without regard to other physical features of the body.

We have had the opportunity of making spectrophotometric examinations of the color of the skin of a group of native American Indians of three distinct tribes. As far as could be determined by physical inspection, or ascertained by direct questioning, these Indians were free from an admixture of white or negro blood. Table 4 presents an analysis of data obtained from reflection curves of the skin of various parts of the body in subjects of both sexes and of different ages.

The American Indian has long been known as a member of the "red" race. This name seems to have been given the race because of the custom of smearing their faces with ochre and not because of any redness in the skin itself. Clinical inspection of the skin of the American Indian suggests a color resembling bronze. As far as is known to us, an attempt has not been made previously to analyze the color of the Indian skin according to accurate standards of color.

The uniformity of results obtained in the data representing fundamental color values, as shown in table 4, is of particular interest. The various areas of skin observed show a degree of brilliance or relative luminosity inversely proportionate to the density of the screen of pigment present. This relationship was demonstrated similarly in an analysis of the other racial types, namely, the white, black and yellow. The hands and face are, of course, much darker than such

TABLE 4  
*Analyses from spectrophotometric data in American Indians*

| Tribe     | Age | Sex         | Area                   | Total excitation | Red      | Green    | Violet   | Hue          | Purity   | Relative luminosity |
|-----------|-----|-------------|------------------------|------------------|----------|----------|----------|--------------|----------|---------------------|
|           |     |             |                        | units            | per cent | per cent | per cent | mili microns | per cent | per cent            |
| Winnebago | 23  | M.          | Chest                  | 4,678            | 44 6     | 33 3     | 22 1     | 590-595      | 41 0     | 26 8                |
|           |     |             | Malar region           | 3,508            | 41 8     | 35 9     | 24 3     | 590-595      | 34 0     | 19 6                |
|           |     |             | Hand above heart level | 2,421            | 42 1     | 33 7     | 24 2     | 590-595      | 34 0     | 13 5                |
|           | 21  | M           | Chest                  | 5,210            | 42 7     | 35 2     | 22 1     | 585          | 41 0     | 29 9                |
|           |     |             | Malar region           | 3,993            | 38 9     | 31 1     | 30 0     | 620          | 11 0     | 20 8                |
|           |     |             | Hand above heart level | 2,050            | 40 4     | 33 7     | 25 9     | 595          | 31 0     | 11 1                |
| Kiowa     | 40  | Step-father | Shoulder (protected)   | 5,394            | 41 2     | 34 2     | 26 6     | 590          | 33 0     | 29 9                |
|           |     |             | Malar region           | 3,268            | 44 1     | 31 1     | 24 8     | 605          | 30 0     | 17 7                |
|           |     |             | Hand at heart level    | 1,067            | 46 5     | 35 7     | 17 8     | 587          | 55 0     | 6 5                 |
|           | 45  | Mother      | Breast                 | 4,622            | 42 8     | 33 1     | 24 1     | 596          | 32 0     | 25 8                |
|           |     |             | Malar region           | 3,770            | 43 5     | 32 3     | 24 2     | 600          | 33 0     | 20 9                |
|           |     |             | Hand at heart level    | 1,905            | 40 5     | 34 2     | 25 3     | 590          | 30 0     | 10 4                |
|           | 18  | Son         | Malar region           | 4,035            | 41 2     | 33 8     | 25 0     | 590          | 30 0     | 22 2                |
|           |     |             | Hand at heart level    | 1,851            | 44 6     | 33 8     | 21 6     | 590          | 43 0     | 10 4                |
|           | 24  | Daughter    | Shoulder (protected)   | 3 015            | 44 8     | 32 9     | 22 3     | 595          | 39 0     | 16 7                |
|           |     |             | Malar region           | 4 217            | 41 5     | 32 9     | 25 6     | 595          | 28 0     | 23 1                |
|           |     |             | Hand at heart level    | 2 377            | 35 8     | 33 5     | 30 7     | 595          | 9 0      | 11 6                |
| Osage     | 32  | Father      | Malar region           | 3 904            | 38 5     | 32 4     | 29 1     | 605          | 15 0     | 20 4                |
|           |     |             | Hand above heart level | 2 480            | 37 3     | 33 3     | 29 4     | 600          | 14 0     | 13 0                |
|           | 27  | Mother      | Malar region           | 3 843            | 39 8     | 31 1     | 29 1     | 620          | 15 0     | 19 9                |
|           |     |             | Hand above heart level | 3,201            | 40 2     | 31 3     | 28 5     | 619          | 17 0     | 17 6                |

TABLE 4—*Concluded*

| Tribe | Age | Sex | Area                | Total excitation |          |          |          | Hue           |          |                     |
|-------|-----|-----|---------------------|------------------|----------|----------|----------|---------------|----------|---------------------|
|       |     |     |                     | units            | Red      | Green    | Violet   |               | Purity   | Relative luminosity |
|       |     |     |                     |                  | per cent | per cent | per cent | milli-microns | per cent | per cent            |
| Osage | 10  | Son | Malar region        | 5,838            | 37 0     | 32 7     | 30 3     | 605           | 11 0     | 29 3                |
|       |     |     | Hand at heart level | 3,045            | 39 2     | 33 9     | 26 9     | 590           | 24 0     | 16 4                |
|       | 7   | Son | Malar region        | 6,117            | 38 0     | 33 5     | 28 5     | 595           | 18 0     | 32 5                |
|       |     |     | Hand at heart level | 2,737            | 39 4     | 34 0     | 26 6     | 590           | 26 0     | 14 9                |

protected areas as the trunk. This would be expected from the difference in habitual exposure to sunlight.

The hue or dominant wavelength throughout the entire series of Indians averaged 593 millimicrons, if the malar areas are excluded. The malar areas in most of the cases examined appeared to contain a little more red, in one case attaining 620 millimicrons. The average was 603 millimicrons. This departure may be because of some anatomic peculiarity of the skin in the malar area, especially as regards its texture, which may allow an undue influence to be exerted on the color of the skin by the superficial capillaries and telangiectasia so commonly found in this region.

The subjects from the Winnebago and Kiowa tribes revealed a level of hue which was similar to that recorded previously for other subjects regardless of race. The values of purity are relatively high except in two instances, namely, in the malar area of the second Winnebago Indian (11 per cent) and in the hand of the fourth Kiowa Indian (9 per cent). In these cases it is possible that the presence of superficial blood may have affected the readings obtained. The relative luminosity or brilliance is uniformly low, as would be expected, since the skin of the Indian, like that of the negro, presents a dark surface and hence reflects little light. In no case did the brilliance exceed 30 per cent in the lightest areas, and in one case (in the hand of the first Kiowa Indian) it dropped to 6.5 per cent, the lowest level of any of our records.

The Osage Indians were a family group and presented a few variations distinguishing them from the other types of Indians, although perhaps not characteristically so. The dominant wavelength lies in the orange region of the spectrum, in one instance at 620 millimicrons. The relative proportions of red, green and violet show a change in the linear relationship by a lowering of the value for red and some-

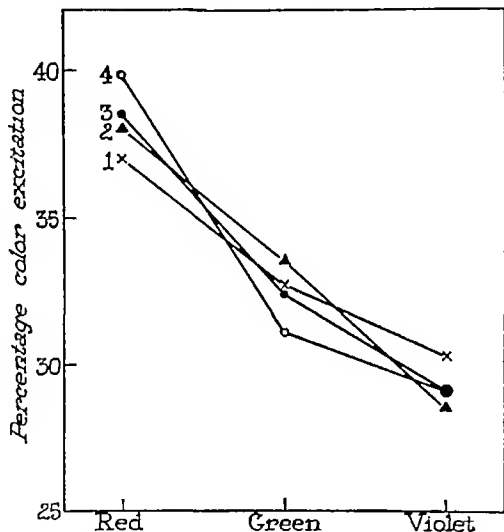


FIG 4 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC DATA IN THE OSAGE INDIAN FAMILY GROUP 1, SON, 2, SON, 3, FATHER, AND 4, MOTHER

times an exaggerated increase in the value for violet. A break in the linear arrangement of these percentage values is reflected, in turn, in the values of the dominant wavelength and purity.

Figure 4 is a graphic representation of the percentage values resulting from an analysis of the reflection curves obtained in the four members of the Osage Indian family. Curves 1, 3 and 4 show a slight

tendency to deviation from a straight line as drawn through the specified value of the green component. This is especially so in curve 4, which is the record of color values from the malar area of the

TABLE 5

*Analyses of spectrophotometric data in subjects presenting pigmentary disturbances*

| Type   | Age | Sex | Area                   | Total excitation | Red      |          |          | Green    |          | Violet   |          | Hue           |          | Purity   | Relative luminosity |
|--|-----|-----|------------------------|------------------|----------|----------|----------|----------|----------|----------|----------|---------------|----------|----------|---------------------|
|  |     |     |                        | units            | per cent | per cent | per cent | per cent | per cent | per cent | per cent | mills microns | per cent | per cent |                     |
| Argyria (confirmed clinically and microscopically) | 40  | M   | Breast                 | 8,094            | 37       | 2        | 34       | 2        | 28       | 6        |          | 586           | 18       | 43       | 0                   |
|  |     |     | Malar region           | 4,398            | 38       | 0        | 34       | 2        | 27       | 8        |          | 587           | 21       | 23       | 5                   |
|  |     |     | Hand at heart level    | 3,064            | 35       | 8        | 33       | 6        | 30       | 6        |          | 590           | 10       | 15       | 5                   |
| Addison's disease                                  | 36  | F   | Chest                  | 5,735            | 36       | 7        | 33       | 4        | 29       | 9        |          | 590           | 12       | 30       | 0                   |
|  |     |     | Malar region           | 3,467            | 41       | 9        | 34       | 0        | 24       | 1        |          | 590           | 34       | 20       | 0                   |
|  |     |     | Hand at heart level    | 2,425            | 40       | 9        | 32       | 4        | 26       | 7        |          | 590           | 25       | 13       | 0                   |
| Arsenism   | 40  | M   | Back (pigmented)       | 5,354            | 41       | 6        | 34       | 9        | 23       | 5        |          | 587           | 36       | 30       | 3                   |
|  |     |     | Malar region           | 4,274            | 42       | 4        | 33       | 2        | 24       | 4        |          | 595           | 32       | 22       | 6                   |
|  |     |     | Hand at heart level    | 2,787            | 43       | 7        | 34       | 5        | 21       | 8        |          | 590           | 41       | 16       | 6                   |
| Arsenism   | 42  | M   | Back (pigmented)       | 5,863            | 42       | 9        | 36       | 0        | 21       | 0        |          | 585           | 45       | 34       | 6                   |
|  |     |     | Forehead               | 5,084            | 43       | 3        | 34       | 0        | 22       | 7        |          | 590           | 40       | 28       | 8                   |
|  |     |     | Hand above heart level | 3,707            | 45       | 7        | 34       | 3        | 20       | 0        |          | 590           | 45       | 21       | 7                   |
| Jaundice   | 43  | M   | Malar region           | 5,156            | 41       | 8        | 33       | 3        | 24       | 9        |          | 595           | 30       | 28       | 4                   |
|  |     |     | Hand above heart level | 5,371            | 37       | 3        | 35       | 4        | 27       | 3        |          | 580           | 24       | 28       | 3                   |

mother of the family. Curve 2 is a straight line, being similar in this respect to all the previous readings obtained on subjects regardless of race or degree of pigmentation. Subsequent readings in other conditions have indicated that the variations presented by the curves 1, 3 and 4 are indicative of the influence exerted by the superficial

blood on the color of the skin. This will be dealt with in greater detail in study III of this series. We may assume that the skin of this group of Osage Indians, being of unusually fine grained and thin texture, allowed greater penetration of the surface blood as a factor in influencing its color.

#### VARIATIONS IN SUBJECTS MANIFESTING ABNORMAL TYPES AND DEGREES OF PIGMENTATION

Further data were obtained on types of pigmentary disturbances in order to permit of a comparison of these data (table 4) with similar analyses of subjects showing normal variation in pigmentation according to exposure to sunlight or to racial type alone. The results are given in table 5.

The argyria was evident on clinical inspection and was of many years' standing. Silver nitrate had been administered for the treatment of ulcer over a period of several years. The diagnosis of argyria was confirmed by histochemical studies on a section of skin removed from the neck. Analysis of the curves obtained does not indicate any marked variations from the gradations of normal pigmentation previously investigated. The dominant wavelength remains at 586 to 590 millimicrons, the percentage of relative luminosity varied according to the amount of habitual exposure of the part, although a value of 43 per cent in the region of the breast indicates lack of pigment, either of melanin or silver. The purity or saturation tends to be fairly low and this may be related to the metallic luster characteristic of argyria.

The values for Addisonian and arsenical pigmentation are not different from those obtained in subjects exhibiting normal variations in pigment. The amount of brilliance recorded by the two cases of arsenism is of interest. The areas of skin on the back were particularly pigmented and were found to be the darkest areas on the body. However, in each case, values were obtained showing a reflection of more than 30 per cent of the total light falling on the surface. This bears out the statement made previously that the eye is a faulty instrument for analyzing the quantity and quality of color.

Records were obtained from two subjects with jaundice and the curves analyzed as in the previous instance. The intensity of the

jaundice was quite pronounced, in each case quantitative estimation of the serum bilirubin by the van den Bergh technic showed 15 to 20 mgm for each 100 cc of blood. The spectrophotometric data in these instances, when analyzed into the fundamental attributes of color, show that jaundice of itself does not alter the normal relations of the color of the skin. The percentages of red, green and violet color and the hue, purity and relative luminosity are practically identical in the subjects with jaundice and in the Japanese and Chinese (table 3). It is reasonable to suppose, however, that degrees of jaundice will reflect themselves in the purity or saturation value of the hue predominating in the skin. However, it is quite evident that the fundamental hue of the skin is not altered by subsequent deposits of bilirubin such as occur in jaundice, without regard to the intensity of the process.

Staining of the tissues of the body by other pigments is occasionally seen after excessive ingestion of such substances as carrots, spinach and egg-yolk, which contain a relatively high percentage of lipochromes such as xanthophyll and carotin. These pigments play an important part in the coloration of leaves and flowers and undoubtedly also contribute the yellow color to animal fat such as that which is present in the subcutaneous tissue. Preliminary investigations on subjects giving evidence of coloration of the skin from such products indicate that the spectrophotometric data obtained will be exactly similar to the records of the subjects with jaundice.

Pigment is one of the few substances which is enjoyed in common by plants and animals. In plants there is a zonal distribution of color depending on the amount of exposure to the direct rays of the sun. In seaweeds, for instance, there is gradation from a green color on the surface, through olive, orange and red as the dimly lighted ocean bottom is approached. This affords an admirable adaptation to environment. It may be true in animals, as in plants, that the pigment on the surface of the body acts in some degree as a regulator of metabolism. The fundamental hue of the human skin remains constant in the spectral region of 590 millimicrons regardless of the amount of habitual exposure of the subject to sunlight. Melanin is laid down as a screen, separating the external environment, on the one hand, from the blood and deeper tissues, on the other. It may

act as a filter in regard to irradiation to which the surface of the body is exposed, possibly barring, by reflection, such fractions of energy as are injurious and unsuitable, and admitting by absorption such portions as are desirable, converting them into a form of energy convenient to immediate service or adaptable to storage for the future needs of the organism

#### CONCLUSIONS

1 Spectrophotometric analysis of the human skin demonstrates certain fairly constant features regarding its color

2 The fundamental hue or dominant wavelength of the skin lies in the spectral region 590 millimicrons (sodium yellow)

3 Deposition of melanin in the skin in response to more or less exposure to sunlight does not disturb the hue or purity of its color but decreases the relative luminosity The more melanin present, the lower the percentage of light reflected from the surface of the skin and the lower the brilliance

4 Pigment is not a racial characteristic. The same hue prevails in the white as in the so-called black, red and yellow races

5 Deposits of pigment in disorders of the skin do not alter the fundamental attributes of its color except as they lower the value for brilliance

6 Jaundice in the skin can be estimated quantitatively but it does not affect the normal values for hue, purity or relative luminosity

#### BIBLIOGRAPHY

- 1 Brunsting, L A , and Sheard, Charles, J Opt. Soc. Am. and Rev Sci Instr , 1929, xvii, 349 A Modification of the Spectrophotometer for Obtaining Measurements on the Reflection of Light from Living Materials
- 2 DORNO, C., Strahlentherapie, 1926, xii, 70 Beiträge zur Kenntnis des Sonnen und Quarzlicht- Erythems und Pigmentes
- 3 Keuffel, Carl, W , J Opt. Soc. Am. and Rev Sci. Instr , 1925, xi, 403 A Direct Reading Spectrophotometer
- 4 Schultze, W , Strahlentherapie, 1926, xxi, 38 Über die Reflexion und Absorption der Haut im sichtbaren Spektrum
- 5 Sheard, Charles, Science 1926, lxxv, 70 Analysis of the Color of the Skin and Its Significance

- 6 Sheard, Charles, and Brown, G E , Arch Int Med , 1926, xxxviii, 816  
The Spectrophotometric Analysis of the Color of the Skin and the Observations by This Method in Normal and in Pathologic Subjects
- 7 Sheard, Charles, and Brunsting, L A , J Clin Invest , 1929, vii, 559 The Color of the Skin as Analyzed by Spectrophotometric Methods I Apparatus and Procedures
- 8 Sonne, Carl, Acta med Scandm , 1920-1921, lrv, 336 The Mode of Action of the Universal Light Bath
- 9 Todd, T W , and Van Gorder, L , Am J Phys Anthropol , 1921, v, 239 The Quantitative Determination of Black Pigmentation in the Skin of the American Negro
- 10 Troland, L T , J Opt Soc Am and Rev Sci Instr , 1922, vi, 527 Report of Committee on Colorimetry of the American Optical Society

# THE COLOR OF THE SKIN AS ANALYZED BY SPECTROPHOTOMETRIC METHODS

## III THE RÔLE OF SUPERFICIAL BLOOD<sup>1</sup>

BY LOUIS A. BRUNSTING AND CHARLES SHEARD

*(From the Section on Dermatology and Syphilology and the Division of Physics and Biophysical Research The Mayo Foundation, Rochester)*

(Received for publication April 29, 1929)

Variation in the superficial blood supply undoubtedly plays a more important rôle in the production of changes in the color of the skin than does an increase or decrease in the pigment. This accounts for the difference in subjects who are fairly well matched otherwise with regard to content of cutaneous pigment. In general, the pigment, in proportion to its amount, prevents the superficial blood from attaining visibility. Changes in color in the brunet, attributable to alterations in the circulation in the skin, are less noticeable than the same changes in the blond, and in the negro they are practically negligible. This holds true for the skin of the same thickness, it being quite obvious that the skin of the palms or soles or other hyperkeratotic areas will permit less light to penetrate than will the thin covering of the malar prominence or the dorsum of the hand.

The color which the blood imparts to the skin is directly related to the amount or quality of the blood present in the peripheral vessels. The size of the arteries, capillaries, and venules varies with the degree of pressure behind them and with the volume of blood to be accommodated. In conditions of hypertension one would expect more force to be exerted peripherally than normally. In the dependent areas of the body, such as the legs and hands, the elements of gravity and stasis of sluggish flow are added. In cases in which the total blood volume

<sup>1</sup> The material in this paper was submitted by Dr. Brunsting to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Dermatology and Syphilology, 1929. Work done in the Division of Physics and Biophysical Research.

and cells are increased, as in polycythemia vera, there is corresponding engorgement of all parts of the vascular tree, including the vessels of the skin, and an attempt is made to establish peripheral collaterals

Deviations from the normal oxygen unsaturation of the hemoglobin, as in cyanosis, cardiac or pulmonary decompensation, carbon monoxide poisoning, methemoglobinemia, and sulphemoglobinemia affect the color of the blood itself macroscopically, and so influence the color of the skin overlying it at any given time Likewise, alterations in the serum content of various dyes, such as bilirubin in jaundice, and xanthophyll and carotin through intestinal absorption, reflect themselves to a certain extent in changes in the color of the skin

Physiologic variations in the area of exposure of surface blood for each unit of surface area of the skin are well known Vasodilation and vasoconstriction occur following changes in the environment, such as heat and cold, and irritation locally by friction, or by the application of medicaments, or again following a disturbance of the emotions, as is seen in blushing or in blanching from fear

Quantitative estimations of the amount of exposed surface of the blood in polycythemia vera have been made by Brown and Sheard (1) by means of physical measurements obtained from projections of instantaneous photomicrographs of minute areas of skin Computations in several cases of polycythemia vera tended to show that the surface capillaries occupied more than three times the allotment of surface area for the normal subject Undoubtedly this accounts for a good deal of the change in color seen in such cases Tintometric studies of color were also made but were not wholly satisfactory

The spectrophotometric measurements which we are presenting aim to show the relative components which enter into the color of the skin when it is subjected to variations in local blood content, either in quantity or quality The water-cell modification of the Keuffel and Esser color analyzer was used in all the cases, special care being given to the area of skin under consideration to avoid extremes of temperature, which might produce local vasodilation or vasoconstriction, and also to avoid undue pressure locally, which might obliterate the superficial vessels to occasion blanching

Sheard and Brown (7) mentioned the presence of absorption bands

occurring constantly in the reflection curves corresponding to the special regions, 630, 580 and 540 millimicrons, respectively. These were interpreted as being due to blood elements, namely, hematin in the tissue cells which would affect the percentage of reflection of red at 630 to 640 millimicrons and oxyhemoglobin at 580 and 540 millimicrons. Such absorption bands occur uniformly throughout the curves obtained in the series reported here. They are most evident in blonds and least evident in the Indians and negro, inasmuch as the superficial blood is more or less obscured by pigment. The band at 630 millimicrons is probably the least constant, being the first to disappear with a decrease in the visibility of the exposed area. The most constant region of change in reflection values is found at 580 millimicrons. There is a sudden decrease in values in the curves of reflection from the yellow to the red regions, with a minimal value at about 580 millimicrons. It is of interest that the dominant wavelength of the normal skin lies at about this point.

Schultze (5), and Dorno (2) also noted this area of lowered reflection values in the general region of 540 to 580 millimicrons. There was more variation in percentage of light reflected with changes in pigmentation in this spectral region than in the red end of the spectrum.

The reflection of light in the violet end of the spectrum was uniformly low in percentage as compared with the red values but not as low as for the region 540 to 580 millimicrons. Schultze noted that this corresponds to the energy distribution curves of Hausser and Vahle (4), and demonstrated radiation of total energy in the violet region of only 0.7 per cent of that radiated in the red region of the spectrum. Other evidence in the literature in regard to the analysis of light reflected by the skin as affected by the presence of blood is singularly lacking.

#### THE REFLECTION OF LIGHT FROM THE SKIN AS AFFECTED BY THE ABSENCE OF BLOOD

Probably the most striking of all color changes in the skin is that which appears after death. We attempted a comparison of the reflection values and their analyses in the living subject with specimens obtained postmortem. An area of skin about 6 by 6 cm. was obtained from the lower part of the chest, in each case soon after death. One

specimen was from a blond, the other from an American Indian. The skin was denuded of fat and blood, spread on a board and stretched to approximately its normal size and held by thumb tacks. The surface was wiped dry and readings were made immediately by placing the specimen against the usual opening in the water-cooled cell.

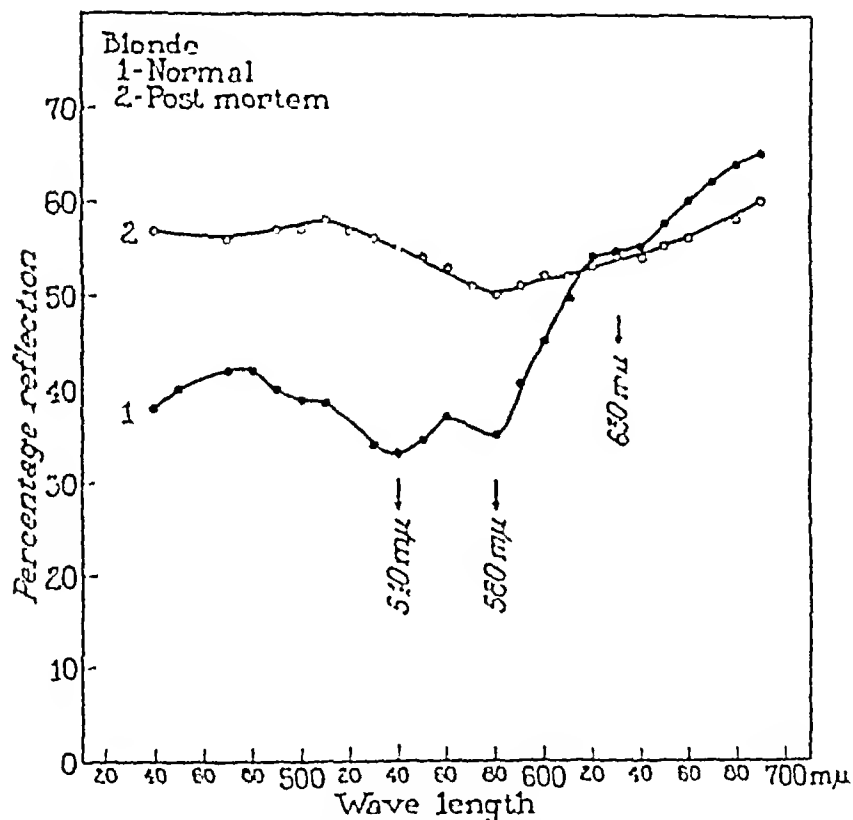


FIG. 1. SPECTROPHOTOMETRIC REFLECTION CURVES. 1, SKIN OF THE CHEST OF A NORMAL LIVING BLOND, COMPARED WITH 2, SKIN OBTAINED POSTMORTEM FROM THE CHEST OF A BLOND OF THE SAME TYPE.

adapted to the color analyzer. For purposes of comparison, records were obtained from a normal blond and a normal Indian of approximately the same body type and body area and of the same sex.

Figures 1 and 2 represent spectrophotometric curves of the reflection values obtained in the normal subject as compared to the con-

ditions postmortem. Curve 1 in each case shows the relative differences in total light reflected by the skin of the blond type as contrasted

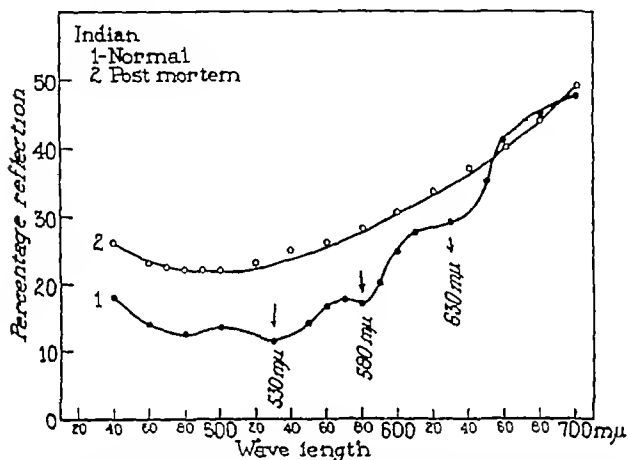


FIG. 2. SPECTROPHOTOMETRIC REFLECTION CURVES. 1, SKIN OF THE CHEST OF A NORMAL LIVING OSAJE INDIAN, COMPARED WITH 2, SKIN OBTAINED POST MORTEM FROM THE CHEST OF AN OSAJE INDIAN OF THE SAME TYPE.

TABLE I

*Analyses from spectrophotometric curves of figures 1 and 2 with a comparison of living blond and Indian skins with specimens obtained postmortem*

| Type       | Total color excitation | Red      | Green    | Violet   | Hue        | Purity   | Brilliance |
|------------|------------------------|----------|----------|----------|------------|----------|------------|
|            | units                  | per cent | per cent | per cent | mμ microns | per cent | per cent   |
| Blond      |                        |          |          |          |            |          |            |
| Living     | 7,339                  | 38.8     | 34.9     | 26.3     | 586        | 27       | 40.0       |
| Postmortem | 9,664                  | 36.3     | 36.3     | 27.4     | 575        | 23       | 51.4       |
| Indian     |                        |          |          |          |            |          |            |
| Living     | 3,015                  | 44.8     | 32.9     | 22.3     | 595        | 39       | 16.7       |
| Postmortem | 4,895                  | 39.7     | 37.2     | 23.1     | 580        | 40       | 27.0       |

to that of the dusky Indian. The reflection values of normal Indian skin reach a figure of 50 per cent in the red end of the spectrum

The irregularities in the normal curves and the absorption regions or spectral bands at 630, 580 and 540 millimicrons, respectively, are to be noted. These are conspicuous by their absence in the curves from

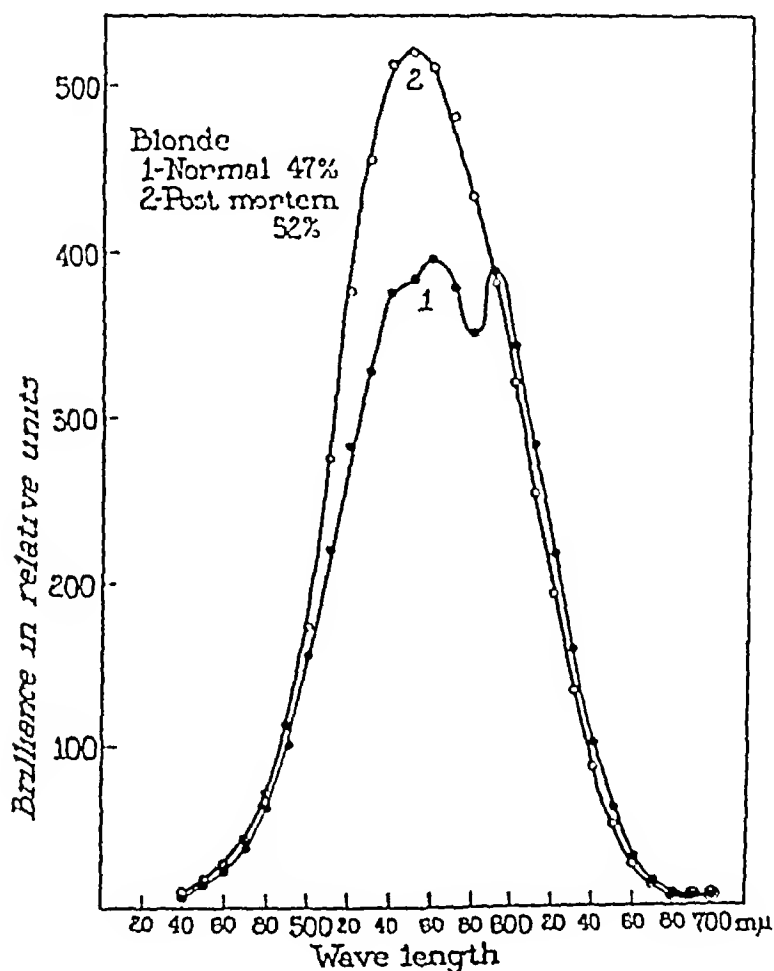


FIG. 3 CURVES OF RELATIVE LUMINOSITY 1, NORMAL BLOND SKIN, AND 2, BLOND SKIN STUDIED POSTMORTEM, TO SHOW VARIATION IN BRILLIANCE

the bloodless skin obtained postmortem. Obliteration of these bands is probably the most striking demonstration possible of the relationship of these absorption regions to the blood elements present in normal skin. There is increased reflection of light in the region, 430 to

580 millimicrons. This is more marked in the case of the light skin than in the pigmented Indian skin, with little absolute change in the region of the orange and red. Analysis of these spectrophotometric curves into color excitation values and their conversion into the attributes of color are shown in table 1.

The total excitation values reveal the extremes of light and dark types and are interpreted in terms of brilliance. The blood in the

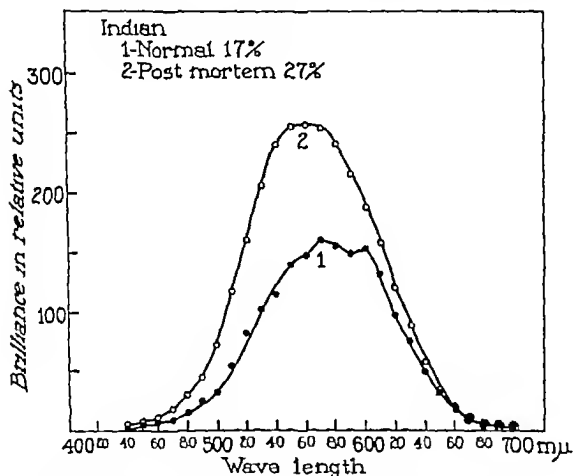


FIG. 4. CURVES OF RELATIVE LUMINOSITY. 1, NORMAL SKIN OF OSAJE INDIAN, AND 2, SKIN OBTAINED POSTMORTEM FROM AN OSAJE INDIAN OF THE SAME TYPE, TO SHOW VARIATION IN BRILLIANCE.

superficial skin acts with the pigment to lower the percentage of light reflected, the blood itself accounting for 10 to 12 per cent of the degree of brilliance, as is seen in figures 3 and 4. The curves obtained from skin free from blood are symmetric and smooth, and those of the living subject tend toward diminished brilliance from wavelength 500 millimicrons toward wavelength 580 millimicrons, with a decided dip in the region of 580 millimicrons (oxyhemoglobin) which is more noticeable in the skin of the blond than in that of the Indian. Decided rearrange-

ment is effected in the distribution of the various percentages of red, green, and violet, as is shown graphically in figures 5 and 6. Curve 1 represents the normal linear arrangement of the summated values

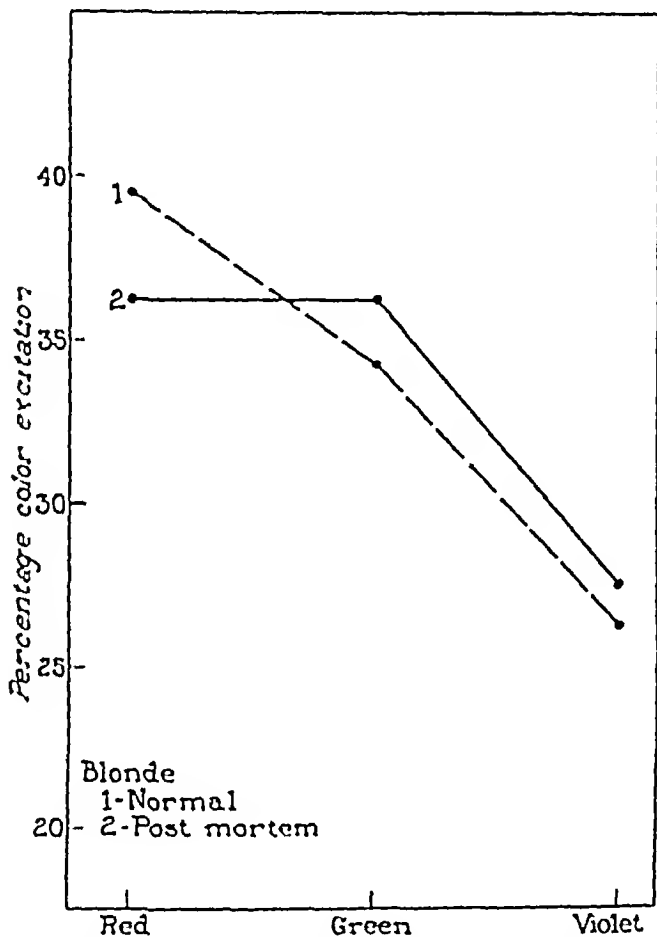


FIG. 5 PERCENTAGE COLOR EXCITATION VALUES 1, SKIN OF NORMAL LIVING BLOND, AND 2, BLOND SKIN STUDIED POSTMORTEM

expressed in percentage form for both the blond and Indian living skins. Curve 2 shows the departure from the linear relationship obtained from an analysis of the same types of skin minus the blood only. The percentage decrease of light reflected in the red with corresponding elevation in the green end is to be noted. The violet is

affected least of all. By plotting these values on the color triangle it will be seen that, although the percentage of purity or saturation remains unchanged in spite of the lack of blood elements, there is a

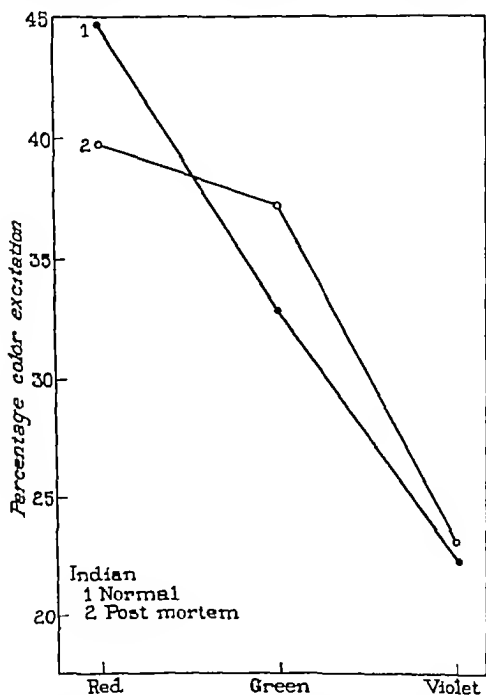


FIG. 6. PERCENTAGE COLOR EXCITATION VALUES 1, SKIN OF NORMAL LIVING OSAGE INDIAN AND 2, SKIN OF OSAGE INDIAN STUDIED POSTMORTEM

shift in the hue or dominant wavelength from the spectral wavelength 586 millimicrons to 575 millimicrons in the blond and from wavelength 595 millimicrons to 580 millimicrons in the Indian.

It has been demonstrated by a comparison of the living with bloodless skin that the superficial blood tends to alter the color of the skin

ment is effected in the distribution of the various percentages of red, green, and violet, as is shown graphically in figures 5 and 6. Curve 1 represents the normal linear arrangement of the summated values

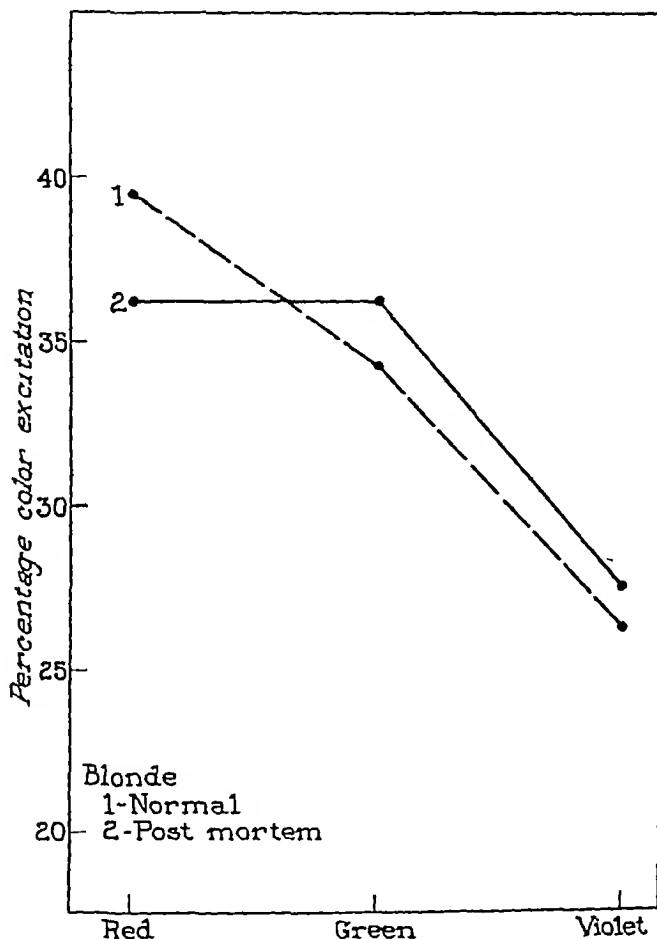


FIG 5 PERCENTAGE COLOR EXCITATION VALUES 1, SKIN OF NORMAL LIVING BLOND, AND 2, BLOND SKIN STUDIED POSTMORTEM

expressed in percentage form for both the blond and Indian living skins. Curve 2 shows the departure from the linear relationship obtained from an analysis of the same types of skin minus the blood only. The percentage decrease of light reflected in the red with corresponding elevation in the green end is to be noted. The violet is

affected least of all. By plotting these values on the color triangle it will be seen that, although the percentage of purity or saturation remains unchanged in spite of the lack of blood elements, there is a

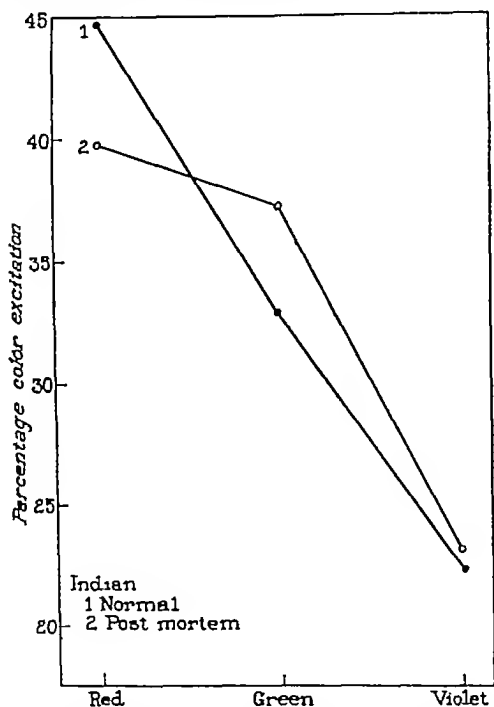


FIG. 6. PERCENTAGE COLOR EXCITATION VALUES 1, SKIN OF NORMAL LIVING OSAGE INDIAN, AND 2, SKIN OF OSAGE INDIAN STUDIED POSTMORTEM

shift in the hue or dominant wavelength from the spectral wavelength 586 millimicrons to 575 millimicrons in the blond and from wavelength 595 millimicrons to 580 millimicrons in the Indian.

It has been demonstrated by a comparison of the living with bloodless skin that the superficial blood tends to alter the color of the skin

by producing elevation in the hue, and a drop in the percentage of relative luminosity without affecting the percentage of purity to any extent

THE REFLECTION OF LIGHT AS AFFECTED BY THE SUPERFICIAL BLOOD  
WITH THE HAND PLACED IN THE DEPENDENT POSITION

Readings were made on the dorsum of the normal hand or the middle or ring finger, with the hand about 25 cm below heart level, using the

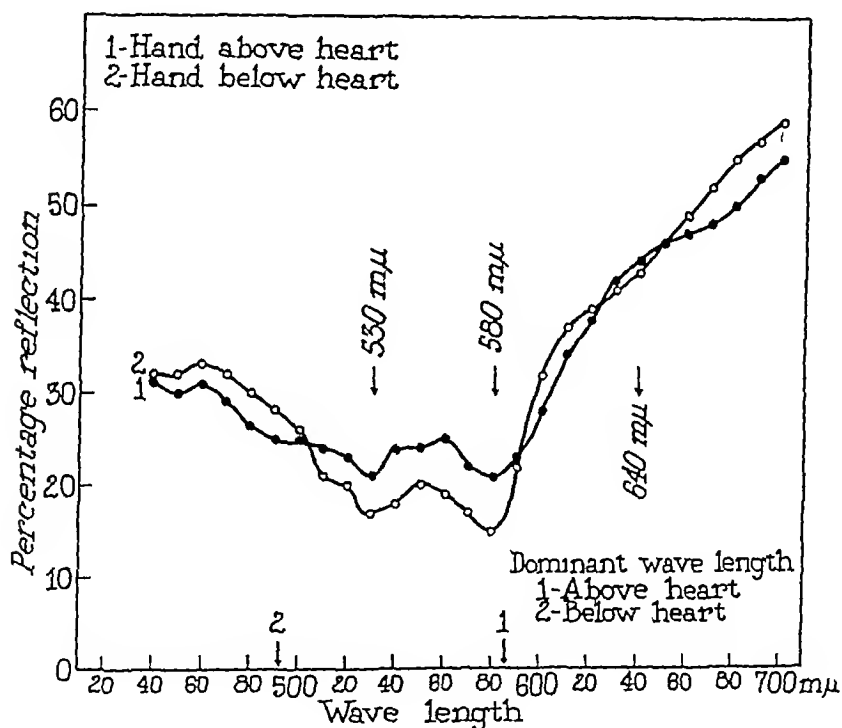


FIG 7 SPECTROPHOTOMETRIC REFLECTION CURVES OF SKIN OF HAND AS PLACED  
IN AN ELEVATED OR DEPENDENT POSITION 1, HAND 25 CM ABOVE  
HEART LEVEL, 2, HAND 25 CM BELOW HEART LEVEL

same areas of skin in each case. The change in color of the hand held in the dependent position depends to some extent on the prominence of the superficial venules, on the time the hand is allowed to remain in this position, and on the amount of interference with the return flow of blood. Cyanosis produced in this fashion has been shown by

Goldschmidt and Light (3) to be due to increased venous pressure peripherally and not related to the degree of oxygen unsaturation of the blood. However, should any stasis develop to retard the flow of the blood, it is reasonable to suppose that the normal relation of carbon dioxide and oxygen in the blood and the tissues would be unbalanced and upset.

Figure 7 shows two spectrophotometric reflection curves of the hand above and below heart level in a moderately brunet type. The increased absorption effect produced in the region 530 milli-

TABLE 2

*Analyses from spectrophotometric curves, illustrating the effect on the various components of color in the skin of placing the hand in the dependent position*

| Case | Type                | Dominant wave-length |                   | Purity            |                   | Relative luminosity |                   |
|------|---------------------|----------------------|-------------------|-------------------|-------------------|---------------------|-------------------|
|      |                     | Above heart level    | Below heart level | Above heart level | Below heart level | Above heart level   | Below heart level |
|      |                     | milli-microns        | milli-microns     | per cent          | per cent          | per cent            | per cent          |
| 1    | Brunet (arsenismus) | 590                  | 592               | 45                | 44                | 21.7                | 23.6              |
| 2    | Negro               | 585                  | 590               | 50                | 33                | 7.5                 | 10.9              |
| 3    | Normal brunet       | 585                  | 600               | 31                | 19                | 30.2                | 22.1              |
| 4    | Normal blond        | 590                  | 600               | 27                | 21                | 42.7                | 35.2              |
| 5    | Japanese            | 587                  | 605               | 31                | 8                 | 24.3                | 18.2              |
| 6    | Chinese             | 585                  | 610               | 28                | 8                 | 27.2                | 26.2              |
| 7    | Normal blond        | 600                  | 620               | 21                | 17                | 36.3                | 26.2              |
| 8    | Marked brunet       | 592                  | 492*              | 28                | 38                | 32.4                | 13.1              |
| 9    | Normal brunet       | 586                  | 493*              | 21                | 18                | 24.7                | 22.9              |

\* Cyanosis

microns and 580 millimicrons when the hand is held below the heart is to be noted. This plays a part in the change in the relations of color factors as determined by analysis of the reflection values in terms of excitation units, as previously described.

Table 2 gives a list of records obtained in a similar manner from a variety of patients. The reflection curves in case 9 are plotted in figure 7.

Table 2 illustrates the variations in dominant wavelength, purity, and relative luminosity brought about by changing of the position of the hand from 25 cm. above the heart to a point 25 cm. below heart.

level. Examples of the most common types of subjects are included. With the hand relatively devoid of superficial blood (above the heart), the dominant wavelength is maintained in the spectral region 590 millimicrons, which corresponds to the region of pure sodium yellow.

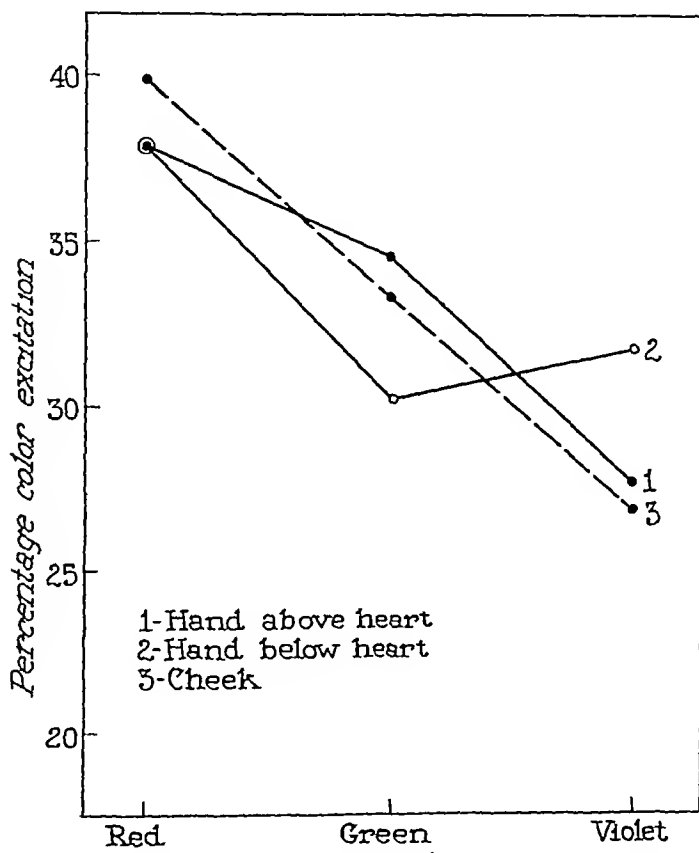


FIG 8 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC DATA 1, HAND ABOVE HEART, 2, HAND BELOW HEART, AND 3, NORMAL CHEEK

If the hand is lowered to a point below heart level, the superficial blood becomes more or less evident and its effect on the color of the skin can be calculated.

In the heavily pigmented subjects (cases 1 and 2), an appreciable change in hue (dominant wavelength) did not take place. Cases

3, 4, 5, 6 and 7 show a shifting toward the red, in one case (case 7) to a dominant wavelength 620 millimicrons. In this case, the normal hue had been established at 600 millimicrons, which may account for the marked rise above the normal variation.

The relation of the color excitation values summated for red, green, and violet in percentage form for case 9 is shown in figure 8. This figure is an analysis of the spectrophotometric reflection curves of figure 7.

Cases 8 and 9 exhibited a change in the dominant wavelength from the normal, 592 to 586 millimicrons, to the blue region at 492 millimicrons. This indicates cyanosis, irrespective of whether or not it is due solely to increased peripheral venous pressure or to changes in the oxygen unsaturation of the blood. Sheard (6), and Sheard and Brown (7) presented similar curves of cyanosis for cases of polycythemia vera following treatment with phenylhydrazine, with shifting of the dominant wavelength to the region 500 millimicrons. Cases 8 and 9 in table 2 show that such a condition is not rare even in normal subjects. The values obtained in each case are directly related to the surface area of superficial blood which attains visibility at any given time. This may occur with a good deal of facility in cases of polycythemia vera and becomes more evident clinically, inasmuch as the entire body is usually involved.

Spectrophotometric and spectroscopic studies of the blood in experimental animals show characteristic absorption bands for hemoglobin in various degrees of oxygen saturation. In viewing the blood coursing through a rabbit's ear, Sonne (8) and others have been able to demonstrate the bands of oxyhemoglobin under normal conditions. Whenever the ear was pinched or the flow of blood was impeded in other ways, the presence of reduced hemoglobin was manifest.

Distention of the superficial venules with blood by means of gravity results, in most instances, in lowering the degree of hue. In other words, the color of the skin possesses a lower percentage of purity or saturation. This is least evident in cases in which the slightest variation in the relative percentages of red, green, and violet are excited, and it is most evident in the two cases of Oriental subjects in table 2 (cases 5 and 6). In these cases the color of the skin approached very nearly an ashen gray, as exemplified by the drop in the purity or

level. Examples of the most common types of subjects are included. With the hand relatively devoid of superficial blood (above the heart), the dominant wavelength is maintained in the spectral region 590 millimicrons, which corresponds to the region of pure sodium yellow.

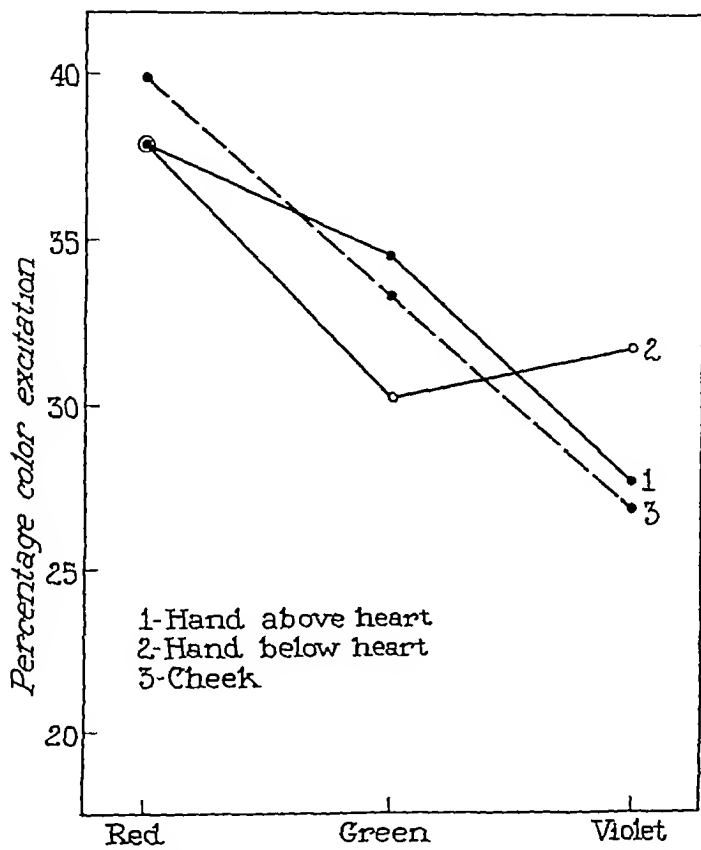


FIG 8 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC DATA 1, HAND ABOVE HEART, 2, HAND BELOW HEART, AND 3, NORMAL CHEEK

If the hand is lowered to a point below heart level, the superficial blood becomes more or less evident and its effect on the color of the skin can be calculated.

In the heavily pigmented subjects (cases 1 and 2), an appreciable change in hue (dominant wavelength) did not take place. Cases

tion curves were obtained from a number of areas of the body which were analyzed into color excitation values and interpreted in terms of the three attributes of color (table 3)

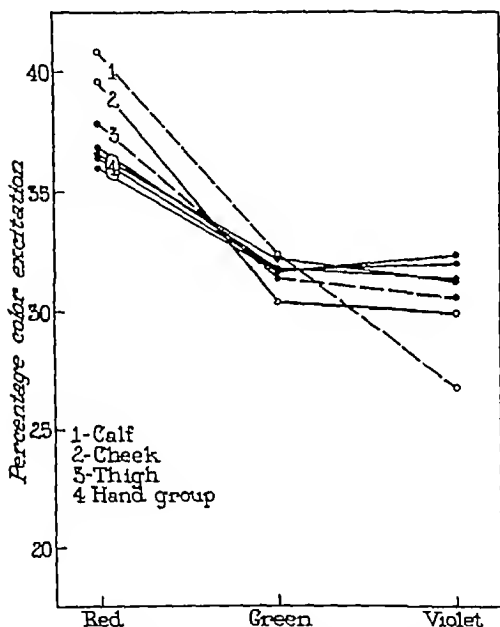


FIG 9 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC DATA FROM A CASE OF DIFFUSE ERYTHRODERMA

Readings made over various areas of the body and with the hand in an elevated and dependent position

The dominant wavelength was unquestionably in the red, being the highest over the malar prominence. In this region it had a value of 660 millimicrons. The hand, when emptied of venous blood by elevation, consistently showed a value of 640 millimicrons. This changed to a cyanotic hue when the hand was held in the dependent position as was shown by the shift from 640 to 493 millimicrons. In this condition the superficial venules filled with blood. The percentage values of

saturation to a level of 8 per cent. In only one case was there an increase in the purity (case 8) and this was associated with marked cyanosis.

The relative luminosity suffers through the presence of superficial blood in proportion to the amount of pigment originally present to mask the effect. The blond subjects manifested the greatest reduction, the negro manifested very little reduction. When cyanosis was induced in one case (case 8) there was a drop in the total brilliance of almost 20 per cent.

TABLE 3

*Analyses of spectrophotometric curves of the skin into the fundamental attributes of color in a case of malignant erythroderma*

| Area              | Dominant wavelength | Purity          | Brilliance      |
|-------------------|---------------------|-----------------|-----------------|
|                   | <i>millimicrons</i> | <i>per cent</i> | <i>per cent</i> |
| Cheek             | 660                 | 12              | 26              |
| Hand              |                     |                 |                 |
| Above heart level | 640                 | 13              | 24              |
| Below heart level | 493                 | 11              | 20              |
| Thigh             | 630                 | 10              | 28              |
| Calf              | 600                 | 22              | 24              |

From a study of the data, it would seem that cyanosis is an end stage of the accumulation of blood in the surface venules, when the flow becomes sluggish through the obstruction introduced by the load of gravity on the column of blood supported. An intermediary stage, or erythrosis, is noted which is similar to a phase of polycythemia vera. It is dependent on the surface area of the blood exposed for each centimeter of surface area and is more or less dependent on the velocity of flow through the part.

#### THE REFLECTION OF LIGHT AS MODIFIED BY CERTAIN DISEASES OF THE SKIN

A few preliminary studies were made of dermatosis in which erythema from peripheral vasodilation was evidently present. Variations from the normal were noted in particular in the following cases.

*Case 1* The patient was aged forty years. The diagnosis was malignant erythroderma (diffuse universal erythema with branny desquamation). Reflec-

Curves 2 and 3 (fig 9) and the group indicated by 4 represent a progressive increase in the percentage of violet until a definite break occurs in the linear relationship. When the percentage of violet excited exceeds the percentage of green there is a shift in the hue from red to blue, which is manifested chiefly in the presence of cyanosis. This was found to be characteristic of the cases of Raynaud's disease and of polycythemia vera following the administration of phenol hydrazine in the report of Sheard and Brown (7).

*Case 2* The patient was aged forty-eight years. The diagnosis was psoriasis vulgaris. Experiments were carried out, with the hand at heart level, on a patch of psoriasis on the calf and an area of skin adjoining the patch. The patient was placed in the prone position, to neutralize the effect of gravity, when the records from the leg were taken. Figure 10 gives spectrophotometric curves of reflection from the three areas which are plotted for purposes of comparison. There is similarity in all the curves, inasmuch as they reveal the decreased percentages of reflection at the regions 530, 580, and 630 millimicrons, respectively. The curve

TABLE 4

*Analyses of spectrophotometric curves of skin from normal areas and an area of psoriasis*

| Area                               | Dominant wavelength | Purity   | Brilliance |
|------------------------------------|---------------------|----------|------------|
|                                    | millimicrons        | per cent | per cent   |
| Normal skin of hand at heart level | 598                 | 19       | 21.0       |
| Normal skin of calf                | 582                 | 32       | 43.3       |
| Psoriatic area of calf             | 620                 | 23       | 22.8       |

from the surface of the patch of psoriasis shows an absorption zone at wavelength 580 millimicrons. This area of skin was denuded of scales so that to the eye the patch was no longer the silvery white so characteristic of psoriasis but possessed the dull red color seen in large plaques over the glabrous surfaces and the trunk. The records obtained from the normal skin of the hand and of the calf were similar except for the difference in the amount of reflection. This is interpreted in the subsequent analysis in terms of brilliance. The hand, being darker than the calf, reflects less light.

Analysis of these spectrophotometric curves into percentage color excitation values is shown in figure 11 and the results obtained after plotting these percentages are expressed in terms of dominant wavelength, purity and brilliance, as shown in table 4.

The values of the dominant wavelength in this case (for the areas of normal skin studied) fell within that which may be termed the low to high normal, that is, from 582 to 598 millimicrons. The hand held at heart level undoubtedly gave a higher reading than when held in an elevated position. The patch of psoriasis was of a reddish hue clinically. This was confirmed spectrophotometrically, since the dominant wavelength proved to be at 620 millimicrons or definitely in

the red The difference in the readings for the three areas may be attributable to the influence of superficial blood in varying amounts, it was least in the velvet-appearing, soft skin of the calf in the prone position, and greatest in the psoriatic patch where the dilated capillaries attain visibility by virtue of the relatively

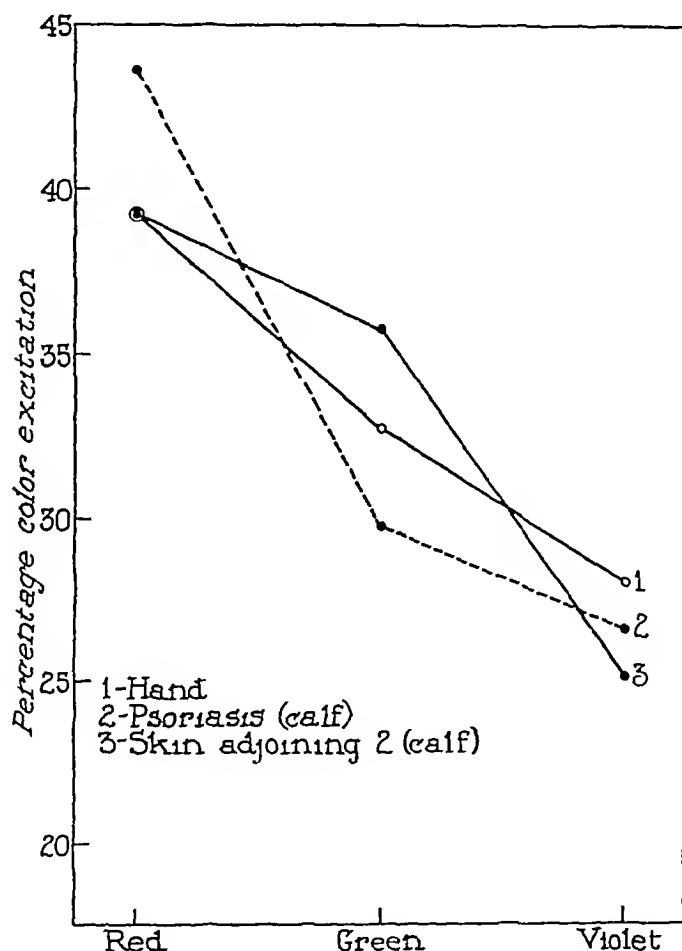


FIG 11 PERCENTAGE COLOR EXCITATION VALUES FROM SPECTROPHOTOMETRIC DATA OF CURVES IN FIGURE 10, 1, HAND AT HEART LEVEL, 2, PSORIATIC PATCH ON CALF, AND 3, SKIN ADJOINING PATCH ON CALF

thin protective covering This is a verification of the clinical sight of "bleeding points" following removal of the thin mica-like scales in psoriasis

The relations of the percentages of red, green and violet color excited in each instance are best shown by figure 11 The normal linear distribution is preserved in curve 1 obtained from the record of the hand Curves 2 and 3 differ in the rela-

tive percentages of red and green, thus altering the slope or the angle of the line plotted, and indicating the paucity of blood in the normal skin of the calf to the engorgement noted over the psoriatic patch

The analysis revealed a difference in percentage in purity in favor of the skin of the calf. This area also proved to be lighter in that there was elevation of the brilliance by 20 per cent, as was expected. The patch of psoriasis was found to be of redder hue and of lower purity and brilliance than the normal skin adjacent to it.

**Case 3** The patient was aged thirty-eight years. The diagnosis was tuberculosis cutis (Sarcoid of Boeck). This patient presented, among other features, areas of diffuse induration of the skin limited to the angles of the jaw and parotid region. These were colored blue, apparently due to the presence of large quantities of venous blood in the dilated superficial blood spaces. Records were made

TABLE 5  
*Analyses of spectrophotometric curves of skin from normal areas and in areas of tuberculosis cutis*

| Area                  | Total color excitation | Red      | Green    | Violet   | Hue          | Purity   | Brilliance |
|-----------------------|------------------------|----------|----------|----------|--------------|----------|------------|
|                       | units                  | per cent | per cent | per cent | millimicrons | per cent | per cent   |
| Normal area on cheek  | 7,077                  | 38.6     | 31.8     | 29.6     | 610          | 13       | 36.7       |
| Lesion on right cheek | 3,683                  | 38.5     | 30.1     | 31.4     | 494*         | 19       | 18.5       |
| Lesion on left cheek  | 3,788                  | 36.9     | 30.6     | 32.5     | 495*         | 16       | 18.9       |
| Hand elevated         | 5,040                  | 38.1     | 32.2     | 29.7     | 610          | 12       | 26.3       |
| Hand dependent        | 3,581                  | 36.7     | 30.9     | 32.4     | 494*         | 15       | 17.0       |

\* Cyanosis.

from each of these areas and also from the normal appearing skin over the malar prominence, as well as from the dorsum of the hand in the elevated and dependent positions. Analyses of the spectrophotometric data in terms of the various components of color are tabulated in table 5.

Comparison of the values obtained from the various areas demonstrated that the colors of the elevated hand and the cheek were identical except for a difference in brilliance of 10 per cent. The dominant wave length at 610 millimicrons was a little nearer the red end of the visible spectrum than is usual for the normal subject, although this would be more likely to be true for the cheek than for the hand. The presence of superficial venous blood was manifested by a lowering in the amount of light reflected (decreased brilliance) and rearrangement of the percentages of red, green, and violet, with a shift in the dominant wavelength from the region of the red to the shorter (blue) spectral value of 494 millimicrons. This is consistently true for the areas in which the disease is found. The presence of the superficial blood tends to diminish the brilliance. The skin of the hand in the

dependent position and the skin over the two areas of lesions on the cheeks gave practically identical spectrophotometric data. This proves beyond doubt that they are of the same color.

### SUMMARY

Quantitative comparisons have been presented showing the variations in the color of the skin in health and disease as dependent on the content of superficial visible blood. Observations on specimens of skin from a blond subject and an Osage Indian obtained postmortem have been compared with records made during life in the same type of subjects to note the effect of the presence or absence of blood on the color of the skin. Furthermore, the effect of a change in the distribution of the superficial blood was observed by placing the hand in an elevated and dependent position in relation to the heart. Various types of dermatosis have been examined spectrophotometrically in order to demonstrate the effect of changes in amount and distribution of the superficial blood on the color of the skin.

### CONCLUSIONS

- 1 The blood in the superficial capillaries exerts a major influence on the color of the skin.
- 2 Spectrophotometric observations of various areas of the human skin demonstrate considerable variation in the amount, quality and distribution of peripheral blood. Diseases of the vascular tree or the blood itself tend to accentuate these changes in the color of the skin.
- 3 The pigment (melanin), in proportion to its density, acts as a screen to prevent the superficial blood from attaining visibility.
- 4 Superficial blood plays a part in the curve of reflection of light from the surface of the skin and is evidenced by absorption zones at the spectral regions, 540, 580 and 630 millimicrons. The absorption band at 630 millimicrons is the least constant, whereas the others persist in all records except in those obtained from specimens of skin devoid of blood (postmortem).
- 5 An abundance of oxygenated blood near the surface of the skin tends to shift the dominant wavelength from the region 590 millimicrons to the orange or red end of the spectrum, reaching a value as high as 620 to 660 millimicrons. There is a diminution of the purity or degree of hue and a lowering of the relative luminosity or brilliance.

6 An abundance of venous blood tends to shift the dominant length toward the blue region of the spectrum (490 to 500 millimicrons) indicating a condition of cyanosis. This is produced by an alteration in the relative percentage values for red, green and violet. No straight line they maintain a linear relationship, but the value for violet is less than the value for green in conditions of marked cyanosis.

7 Spectrophotometric records and analyses may assist in the delineation of changes due to combined alterations in the content of hemoglobin in the blood and pigment in the skin of human beings.

#### BIBLIOGRAPHY

- 1 Brown, G. E., and Sheard, Charles, *J. Clin. Invest.*, 1926, **li**, 423. Observations on the skin capillaries in cases of polycythemia vera and the effect of these capillaries in the production of erythrosis.
- 2 Dorn, C., *Strahlentherapie*, 1926, **xxii**, 70. Beiträge zur Kenntnis des Erythems und Quarzlicht Erythems und Pigmentes.
- 3 Goldschmidt, Samuel, and Light, A. B., *Am. J. Physiol.*, 1925, **lxx**, 15. A peripheral cyanosis, unrelated to oxygen unsaturation, produced by increased peripheral venous pressure.
- 4 Hausser, K. W., and Vahle, Wilhelm, *Strahlentherapie*, 1922, **xlii**, 4. Abhängigkeit des Lichterythems und der Pigmentbildung von der Wellenzahl (Wellenlänge) der erregenden Strahlung.
- 5 Schultze, Walther, *Strahlentherapie*, 1926, **xxii**, 38. Über die Reflexion und Absorption der Haut im sichtbaren Spektrum.
- 6 Sheard, Charles, *Science*, 1926, **lxiv**, 70. Analysis of the color of the skin and its significance.
- 7 Sheard, Charles, and Brown, G. E., *Arch. Int. Med.*, 1926, **xxxviii**, 816. Spectrophotometric analysis of the color of the skin and the observation of this method in normal and in pathologic subjects.
- 8 Sonne, Carl, *Acta med. Scand.*, 1921, **lv**, 336. The mode of action of the universal light bath.



## STUDIES ON RED BLOOD CELL DIAMETER

### III THE RELATIVE DIAMETER OF IMMATURE (RETICULOCYTES) AND ADULT RED BLOOD CELLS IN HEALTH AND ANEMIA, ESPECIALLY IN PERNICIOUS ANEMIA

By ELBERT LAPSLEY PERSONS

*(From the Medical Service of the Collis P Huntington Memorial Hospital of Harvard  
University and the Thorndike Memorial Laboratory of the Boston City Hospital)*

(Received for publication April 15 1929)

It has been shown (1) (2) (4) that curves of the frequency of the diameters of normal erythrocytes are symmetrical and uniform. In most cases of anemia classed as secondary, frequency curves of measurement of the red blood cell diameters are displaced to the left, or small side, of the normal and the mean diameter of the cells is less than normal. In contrast to this, in pernicious anemia in relapse the frequency curve usually is displaced to the right and the mean diameter is greater than normal. In marked remissions of pernicious anemia induced by the feeding of suitable amounts of liver or potent liver extracts diameter frequency curves often approach normal (3) (9).

The irregularities of the frequency curves of the diameters of erythrocytes from pernicious anemia has aroused interest and Price-Jones (5) has suggested that in view of the apparent skewness of these curves there may be three elements of red blood cell formation in the bone marrow, the normal, giving the basic curve, together with the smaller cells (anemic) and larger (pernicious) cells which impart the skewness. It was suggested to me by Dr. George R. Minot that a study of the relative diameter of the young blood corpuscles (reticulated red blood cells, reticulocytes) to adult red blood cells might throw light on the nature of the skewness and that such observations upon normal red blood cells and those from various types of anemia might give information concerning blood formation. Heretofore no especial attention appears to have been given to the actual or compara-

tive sizes of the reticulocytes under varying conditions Hawes (6) in 1909, described them as uniformly larger than non-reticulated cells Minot and Lee (10) and Key (7) have noted the occurrence of small reticulocytes Gram (8) writes that "vital-stainable corpuscles" are "relatively thick elements" and "may dominate the picture in periods of forced regeneration of simple anemias and in hemolytic jaundice" Knowledge concerning the growth of tissue cells has been obtained by determinations of the nuclear-cytoplasmic ratio and this favored the idea that a comparison of the size of the mature and immature erythrocytes might help to explain certain features of the formation of red blood corpuscles Twenty-six observations of this sort have been made of the erythrocytes of 5 normal and 19 pathological bloods, particularly from cases of pernicious anemia, and this paper concerns the results obtained

#### METHOD

Measurements of the red blood cell diameters were made from some specimens of capillary and some of venous blood, stained supravitaly with brilliant cresyl blue between two thin glass coverslips The blood was then stained with Wright's stain and the preparations mounted The method of measuring the diameters was the same as that described in previous papers of this series (2) (3) To obtain adult red blood cell diameters 250 consecutive cells having distinct boundaries were measured Adult red blood cells were considered to be those with no intracellular structures such as reticulum, Howell-Jolly bodies, Cabot ring bodies or refractile granules (Isaacs) The large, indistinct, pale red blood cells occurring in occasional fields of pernicious anemia blood were also omitted on the ground that they probably represent changes occurring after the cells are formed rather than abnormalities in blood formation They are so rare that their inclusion would have changed nothing of importance in the data except the upper limit of red blood cell size The occurrence of polychromatophilia may be taken to indicate improper staining by the method used Hence to be as certain as possible that all reticulocytes were stained, no preparation containing polychromatophilic cells without reticulum was used for measurements The 100 reticulocytes measured were found among or close to the adult red blood cells which had

been measured, so that, irrespective of absolute values, the relative values for adult red blood cell and reticulocyte diameters should be of significance

The absolute variations in the diameters of the red blood cells from venous and capillary blood were found to be very slight and no greater than those of the red blood cells from two different preparations of the same capillary blood taken at the same time. Furthermore, the distribution of red blood cell diameters and the relations of reticulocyte to adult red blood cell diameters were practically identical in the two types of blood.

The mean or average diameter was computed arithmetically from the original data. The data were plotted for study as frequency curves and also as summation frequency curves on Whipple's arithmetic probability paper (see Fig. 2) which converts a frequency curve showing perfect adherence to the "law of probability" into a straight line. The numerical ratio between mean reticulocyte and mean adult red blood cell diameter was computed for each case and is included in the data presented.

#### COMPARISON OF RETICULOCYTES AND ADULT RED BLOOD CELLS

Figure 1 demonstrates by frequency curves the size relationships found to exist between the diameters of adult red blood cells and reticulocytes in normal bloods and in a case of pernicious anemia in relapse. A comparison of the curve for the percentage distribution of the diameters of 1250 adult red blood cells from five normal bloods with that for 500 reticulocytes from the same bloods indicates that the distribution of sizes is strikingly similar, but that the reticulocytes in normal bloods are uniformly somewhat larger than the adult red blood cells. Since normal bloods contain only about one per cent of reticulocytes this curve for adult red blood cells very nearly corresponds with comparable ones for all red blood cells of normal blood which have been published (1) (2) (4) (8).

The case of pernicious anemia recorded as case 18, table 1, for which data are charted in figure 1 had a red blood cell count of 1.8 million per cu mm and a typical blood picture of that disease. The measurements are similar in all respects to those of the cells from the other nine pernicious anemia patients studied with low red blood cell

counts The frequency curve (fig 1) for the diameters of the 250 adult red blood cells measured is similar to other diameter frequency curves which have been published (2) (3) (4) (5) for all cells in com-

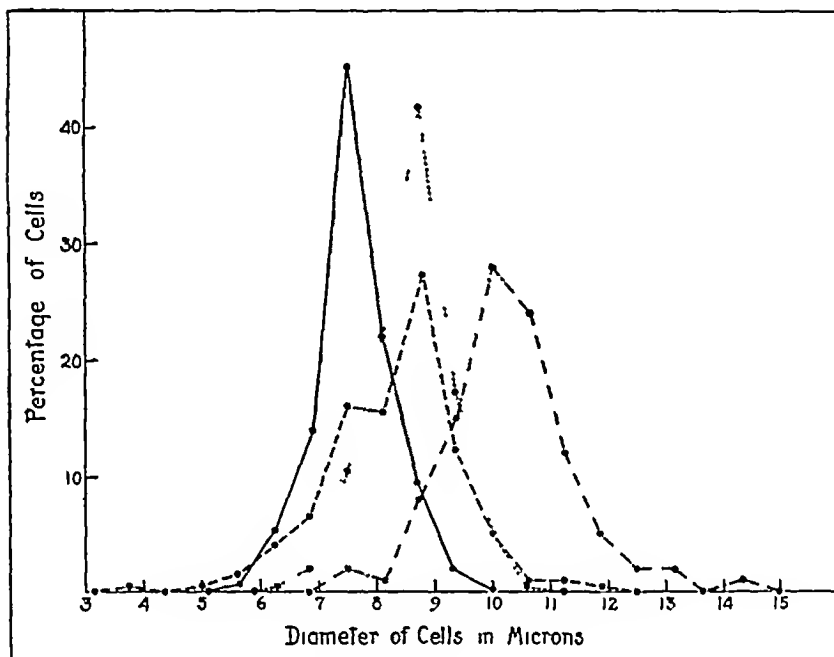


FIG 1 FREQUENCY CURVES OF THE SIZE RELATIONSHIPS BETWEEN THE DIAMETERS OF RETICULOCYTES AND ADULT RED BLOOD CELLS IN NORMAL BLOODS AND IN A CASE OF PERNICIOUS ANEMIA

The curves for normal bloods are constructed from the measurements of 1250 adult red blood cells and 500 reticulocytes from 5 individuals. The curves for the cells from a case (no 18, table 1) of pernicious anemia are constructed from the measurement of 250 adult red blood cells and 100 reticulocytes.

- Normal adult red blood cells
- Normal reticulocytes
- - - Pernicious anemia adult red blood cells
- - - Pernicious anemia reticulocytes

parable cases of pernicious anemia. A comparison of this curve with that for 100 reticulocytes from the same blood shows a similarity of shape which, allowing for the smaller number of cells measured, is

quite as striking as that between the diameter frequency curves for reticulocytes and adult red blood cells from normal bloods

Table 1 shows the results of measurements of adult red blood cells and reticulocytes from 24 persons. These may be grouped in three classes, normal (5) "secondary" anemia due to various causes, (10) and pernicious anemia (9)

#### *A Normal bloods*

Cases 1 to 5 were from healthy individuals who had normal blood pictures and the figures for their adult red blood cells conform fairly well to those obtained by other investigators for normal red blood cells. It is recognized that the adult red blood cell size of case 3 is lower than that of any normal blood which has been reported in this series of papers. The specimen was from a young adult male on whom repeated blood examinations have been made during the past four years without any abnormalities having been detected.

Normal reticulocytes average about 1 micron larger in diameter than normal adult red blood cells. The average of the means is 8.6 microns for the reticulocytes as against 7.6 microns for the adult red blood cells. The ratio of mean reticulocyte diameter to mean adult red blood cell diameter varies from 1.12 to 1.15. The frequency curves obtained by plotting the averaged data from these 5 normal bloods are shown in figure 1. Summation-frequency curves of reticulocyte and adult red blood cell diameters from these bloods are so nearly straight that nothing but errors of measurement could be postulated to explain deviations and in each case the reticulocyte diameter distribution curve is almost parallel to that for adult red blood cell diameter.

#### *B "Secondary" anemia*

In the cases of "secondary" anemia the reticulocyte-adult red blood cell diameter ratio (table 1) is lower than in the normal individuals.

The measurements marked 4b and 5b (table 1) were made on cells taken from normal individuals (cases 4 and 5) three days after the loss of from 500 to 600 cc. of blood, while measurements labelled 4a and 5a were made just before the blood was removed. In each case the red blood cells had fallen approximately half a million per cubic millimeter

TABLE 1  
The diameter of adult and reticulated red blood cells  
Normal blood

| Case | Adult red blood cells           |               |               |         |                 | Reticulocytes      |                  |               |               | Differ-<br>ence of<br>means | Ratio*<br>Reticulocytes<br>Adult |
|------|---------------------------------|---------------|---------------|---------|-----------------|--------------------|------------------|---------------|---------------|-----------------------------|----------------------------------|
|      | Red blood<br>cells<br>per cu mm | Lower<br>size | Upper<br>size | Spread  | Disper-<br>sion | Median<br>diameter | Mean<br>diameter | Lower<br>size | Upper<br>size | Spread                      | Mean<br>diameter                 |
|      | millions<br>per cu mm           | microns       | microns       | microns | microns         | microns            | microns          | microns       | microns       | microns                     | microns                          |
| 1    | 4.7                             | 5.0           | 9.4           | 4.4     | 1.1             | 8.1                | 7.8              | 6.9           | 10.6          | 3.7                         | 8.8                              |
| 2    | 4.9                             | 6.3           | 10.0          | 3.7     | 1.0             | 7.9                | 7.7              | 6.9           | 10.0          | 3.1                         | 8.7                              |
| 3    | 4.9                             | 6.3           | 8.8           | 2.5     | 1.2             | 7.4                | 7.0              | 6.3           | 9.4           | 3.1                         | 8.0                              |
| 4a   | 5.0                             | 6.3           | 9.4           | 3.1     | 1.1             | 8.0                | 7.7              | 7.5           | 10.0          | 2.5                         | 8.8                              |
| 4b   | 4.5                             | 6.3           | 10.0          | 3.7     | 1.2             | 8.0                | 7.9              | 7.5           | 10.6          | 3.1                         | 8.7                              |
| 5a   | 4.8                             | 6.3           | 10.0          | 3.7     | 1.2             | 8.0                | 7.8              | 8.1           | 11.3          | 3.2                         | 9.0                              |
| 5b   | 4.3                             | 6.3           | 10.0          | 3.7     |                 |                    | 7.8              | 7.5           | 11.3          | 3.8                         | 8.9                              |

"Secondary" anemias

| Case | Adult red blood cells    |               |               |         |                  | Reticulocytes |               |         |                  | Differ-<br>ence of<br>means | Ratio*<br>Reticulocytes<br>Adult | Causes of anemia              |
|------|--------------------------|---------------|---------------|---------|------------------|---------------|---------------|---------|------------------|-----------------------------|----------------------------------|-------------------------------|
|      | Red blood<br>cells       | Lower<br>size | Upper<br>size | Spread  | Mean<br>diameter | Lower<br>size | Upper<br>size | Spread  | Mean<br>diameter | microns                     |                                  |                               |
|      | millions<br>per<br>cu mm | microns       | microns       | microns | microns          | microns       | microns       | microns | microns          | microns                     |                                  |                               |
| 6    | 4.3                      | 5.6           | 9.4           | 3.8     | 7.5              | 6.9           | 9.4           | 2.5     | 8.2              | 0.7                         | 1.10                             | Hemorrhage, chronic           |
| 7    | 2.0                      | 5.6           | 10.6          | 5.0     | 8.0              | 6.9           | 11.3          | 4.4     | 8.6              | 0.6                         | 1.08                             | Hemorrhage, acute             |
| 8    | 2.8                      | 7.0           | 10.6          | 5.0     | 7.7              | 6.3           | 10.6          | 4.3     | 8.3              | 0.6                         | 1.07                             | Hemorrhage, acute             |
| 9    | 1.8                      | 20.0          | 5.0           | 10.6    | 7.4              | 6.3           | 11.3          | 5.0     | 8.1              | 0.7                         | 1.10                             | Repeated hemorrhage           |
| 10   | 3.2                      | 17.0          | 4.6           | 9.9     | 6.7              | 5.0           | 10.0          | 5.0     | 7.3              | 0.6                         | 1.09                             | Congenital hemolytic jaundice |
| 11   | 3.5                      | 12.5          | 5.0           | 4.4     | 7.0              | 6.3           | 9.4           | 3.1     | 7.6              | 0.6                         | 1.09                             | Congenital hemolytic jaundice |
| 12   | 2.2                      | 5.0           | 10.0          | 5.0     | 7.2              | 6.3           | 10.6          | 4.3     | 8.0              | 0.8                         | 1.10                             | Chronic myelogenous leukemia  |
| 13   | 3.2                      | 21.0          | 4.4           | 10.6    | 8.0              | 6.9           | 10.6          | 3.7     | 8.4              | 0.4                         | 1.06                             | Methemoglobinemia             |
| 14   | 1.6                      | 15.5          | 5.0           | 9.4     | 7.2              | 5.6           | 10.0          | 4.4     | 7.6              | 0.4                         | 1.06                             | Cancer                        |
| 15   | 1.2                      | 2.3           | 8.8           | 5.0     | 7.0              | 5.0           | 11.3          | 6.3     | 7.4              | 0.4                         | 1.06                             | Cancer                        |

Pernicious anemia (with low red blood cell counts)

| Case | Red blood cells<br>millions<br>per c.c. | Hemo-<br>globin<br>per cent | Reticu-<br>locytes<br>per cent | Adult red blood cells |               |         |                  | Reticulocytes |               |         |                  | Differ-<br>ence of<br>means | Ratio<br>Reticulocytes<br>Adult |
|------|---|-----------------------------|--------------------------------|-----------------------|---------------|---------|------------------|---------------|---------------|---------|------------------|-----------------------------|---------------------------------|
|      |   |                             |                                | Lower<br>size         | Upper<br>size | Spread  | Mean<br>diameter | Lower<br>size | Upper<br>size | Spread  | Mean<br>diameter |                             |                                 |
|      |   |                             |                                | microns               | microns       | microns | microns          | microns       | microns       | microns | microns          | microns                     |                                 |
| 16   | 1.4                                     | 20                          | 0.8                            | 3.8                   | 12.5          | 8.7     | 8.2              | 7.5           | 15.0          | 7.5     | 10.6             | 2.4                         | 1.29                            |
| 17   | 1.2                                     | 47                          | 0.3                            | 3.8                   | 12.5          | 8.7     | 8.0              | 5.6           | 13.8          | 8.2     | 10.1             | 2.1                         | 1.25                            |
| 18   | 1.8                                     | 48                          | 1.4                            | 3.8                   | 11.9          | 8.1     | 8.4              | 7.5           | 14.4          | 6.9     | 10.3             | 1.9                         | 1.23                            |
| 19   | 2.1                                     | 47                          | 0.9                            | 5.0                   | 12.5          | 7.5     | 7.9              | 6.9           | 12.5          | 5.6     | 9.9              | 2.0                         | 1.25                            |
| 20   | 1.2                                     | 37                          | 3.6                            | 3.8                   | 12.5          | 8.7     | 8.4              | 6.3           | 15.0          | 8.7     | 10.6             | 2.2                         | 1.26                            |
| 21   | 2.2                                     | 61                          | 0.3                            | 4.4                   | 11.9          | 7.5     | 8.5              | 8.1           | 13.8          | 5.7     | 10.6             | 2.1                         | 1.21                            |
| 22   | 0.6                                     | 17                          | 8.3                            | 5.0                   | 11.9          | 6.9     | 8.0              | 6.3           | 13.8          | 7.5     | 10.2             | 2.2                         | 1.28                            |
| 23   | 2.0                                     | 47                          | 6.4                            | 5.0                   | 11.9          | 6.9     | 8.0              | 6.9           | 13.1          | 6.2     | 10.1             | 2.1                         | 1.27                            |
| 24   | 1.6                                     | 36                          | 1.8                            | 5.0                   | 11.3          | 6.3     | 8.5              | 8.1           | 16.9          | 8.8     | 10.4             | 1.9                         | 1.22                            |

\* The second decimal place is omitted in the table but was used to determine the ratio  $\frac{\text{Reticulocytes}}{\text{Adult}}$

and about 4 per cent of reticulocytes were present indicating a response of the bone marrow. It is interesting that there is a slight increase in the adult red blood cell diameter in case 4 and no change for this value in case 5, while in both cases the reticulocytes are smaller than before the loss of blood so that the reticulocyte adult red blood cell diameter ratio dropped from 1.15 to 1.10 in case 4 and from 1.15 to 1.13 in case 5. A larger series of similar cases must be studied before the importance of these changes can be properly evaluated.

The anemia in cases 6 to 9 was due to hemorrhage. Case 6 occurred in a woman who had had menstrual disturbances with abnormal blood loss and a slight anemia for several months before the specimen was taken. The mean adult red blood cell diameter while within normal limits, is smaller than that of most normal bloods, and the difference, 0.7 micron, between the mean reticulocyte and adult red blood cell diameters is definitely decreased, giving a reticulocyte-adult red blood cell diameter-ratio of 1.10. Cases 7 and 8 occurred in patients who had had a single severe hematemesis within a week of the time the specimen was taken. In these cases the mean adult red blood cell diameter is not abnormal, but the distribution has been changed, as is indicated by the increased value for the "spread". The spread of the reticulocytes is also somewhat increased. Again the differences between the mean reticulocyte and adult red blood cell diameters are reduced, so that the ratios between them are 1.08 and 1.07, but again the response of the bone marrow was slight. Case 9 occurred in a patient who had had repeated severe hematemeses during the five weeks previous to the day the specimen was taken, and while the "spread" of the adult red blood cells is definitely increased the mean adult red blood cell diameter is quite small. The "spread" of the reticulocyte diameters is also increased and the mean reticulocyte diameter is correspondingly reduced so that the reticulocyte-adult red blood cell diameter ratio is 1.10, the same as that of the milder prolonged anemia due to blood loss (case 6).

Cases number 10 to 15 inclusive were of secondary anemia from causes other than hemorrhage. Case 10 and 11 were of congenital hemolytic jaundice and were both severe enough to justify splenectomy. The measurements of the cells from these two bloods are strikingly similar and resemble those reported by Whitcher (11), both retic-

ulocyte and adult red blood cell "spreads" being increased and both sets of mean diameters being decreased considerably, so that the reticulocyte-adult red blood cell diameter ratio is 1.09<sup>1</sup>

Case 12 was an example of "secondary" anemia accompanying chronic myelogenous leukemia and the measurements are very similar to those of case 9 with anemia from acute blood loss

Case 13 exhibited varying degrees of anemia associated with proved methemoglobinemia existant for about eighteen months. At the time the specimen was taken the patient's red blood cell count was increasing and was about 2.5 million per cubic millimeter. The measurements of adult red blood cell diameters fail to show decreased mean cell size, but the "spread" is markedly increased. The many reticulocytes (21 per cent) are relatively small and the ratio of the mean diameters of the reticulocytes and adult red blood cells is low (1.06)

Case 14 showed a severe anemia due to cancer. The blood showed a high reticulocyte count, nucleated red blood cells and much poikilocytosis. The measurements show small adult red blood cells and reticulocytes and the ratio between their sizes is reduced to 1.06

Case 15 was the most interesting single case of the series. A blond Norwegian of 53 had severe anemia of about three months' duration. His history and physical examination suggested that he had pernicious anemia. He was transfused twice with blood and liver feeding commenced, but he died four days after the first transfusion. At autopsy the cause of death proved to be a scirrhus carcinoma of the stomach with metastases to the regional lymph nodes, liver and bone marrow. The measurements of the red blood cells after the first transfusion showed marked reduction in the mean adult red blood cell diameter and more than proportionate reduction in the mean reticulocyte diameter so that the ratio between them was reduced to 1.06. Until the time of death one of the chief findings which weighed against the diagnosis of pernicious anemia was these measurements of his red blood cell diameters.

Frequency curves plotted from measurements of the diameters of adult red blood cells in "secondary" anemia are similar to those

<sup>1</sup> An unpublished case of congenital hemolytic jaundice showing an increase in the mean diameter of the reticulocytes has been observed by Means and Thomas

obtained by other observers for all types of cells in similar bloods and the curves for reticulocyte diameters show corresponding shapes, but are not displaced as far to the right as are these curves for normal bloods. Summation-frequency curves on arithmetic probability paper are nearly straight for both reticulocyte and adult red blood cell diameters, if enough cells are measured to overcome the effect of the increased variation in size ("spread") found in "secondary" anemia bloods. Again the curve for reticulocyte diameter frequency is displaced to the right less than in normal bloods.

### *C Pernicious anemia*

Measurements from 9 cases of pernicious anemia with low red blood cell counts (cases 16-24, table 1) show that in every instance the mean red blood cell diameter is large, as is also the spread of both adult red blood cells and reticulocytes. The mean adult red blood cell diameter is close to normal in cases 19, 22 and 23, but the mean reticulocyte diameter is distinctly above normal. Since the mean reticulocyte diameter is relatively very large, the ratios of the mean diameters are from 1.21 to 1.29, or not less than 0.06 above the highest ratio obtained in a normal blood.

Frequency curves of adult red blood cell diameter measurements from these bloods are similar to those reported for pernicious anemia bloods by other observers (2) (3) (4) (5), and the reticulocyte diameter frequency curves correspond in shape to those of adult red blood cells from the same bloods, but are displaced much farther to the right than are those from normal bloods.

Summation-frequency curves plotted on arithmetic probability paper of 250 red blood cells from pernicious anemia bloods show deviations from the straight line plot for normal bloods, but in two cases in which 1000 cells of each were measured the resulting plots were as nearly straight as are corresponding curves from normal bloods.

Figure 2 shows the summation diameter frequency curve obtained from the measurement of 1000 adult red blood cells from case 20, and a similar curve of diameter measurements of 100 reticulocytes from the same blood. These two curves are almost parallel and bear the same relation to each other that corresponding curves for red blood cells from normal bloods and secondary anemias do, except that the curve

for reticulocyte diameters is displaced farther to the right. This very striking difference between the sizes of reticulocytes and adult red blood cells, which is demonstrated both by the figures and the curves for each of the nine cases of pernicious anemia with a low blood cell count and which is much less marked in all the other cases studied may be characteristic of the blood picture of this disease.

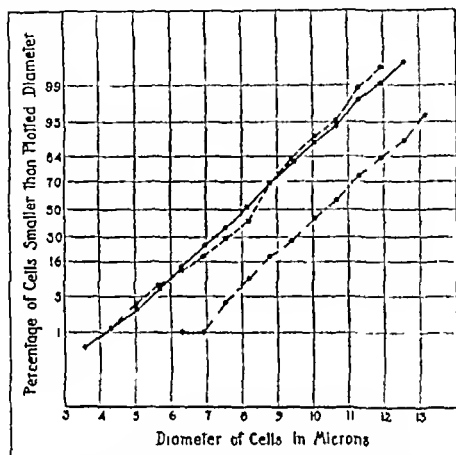


FIG. 2. PERNICIOUS ANEMIA. SUMMATION DIAMETER FREQUENCY CURVES OF 1000 ADULT RED BLOOD CELLS, 250 ADULT RED BLOOD CELLS AND 100 RETICULOCYTES FROM A CASE OF PERNICIOUS ANEMIA PLOTTED ON ARITHMETIC PROBABILITY PAPER.

The curves for adult red blood cells show that the skewness of the curve based on only 250 cells disappears when a large number of cells (1000) are measured.

- 1000 adult red blood cells
- - - - 250 adult red blood cells
- . - . 100 reticulocytes

#### DISCUSSION

The striking parallelism between the adult red blood cell and reticulocyte diameter distribution curves furnishes further evidence that the formation of reticulocytes is most intimately connected with

the formation of adult red blood cells. In considering the relation of reticulocytes to adult red blood cells, there are two obvious possibilities. One is that the reticulocyte in the peripheral blood is a young red blood cell which will lose its reticulum in a few days, shrink somewhat in size, and become an adult cell. The other possibility is that reticulocytes are liberated from the marrow somewhat prematurely, and that they remain in the peripheral blood as such until dissolution takes place, but that the rate of their liberation is approximately proportionate to the rate of liberation of mature red blood cells. The present studies do not lead to deductions in favor of either of these hypotheses. The former is much the most probable one because one may observe extrusion and loss of reticular material in cells kept in plasma outside the body at 37.5°C. The investigation does indicate that a close correlation exists between the sizes of reticulocytes and adult red blood cells in normal and pathological bloods.

The measurements from the twelve cases of "secondary" anemia recorded in table 1 indicate that when an extra load is thrown upon the bone marrow by "chronic" hemorrhage or red blood cell destruction it responds by throwing out reticulocytes which are relatively smaller than normal reticulocytes. (The actual measurements are smaller in only a fraction of the cases.) This is not what one would expect if the response of the bone marrow were simply one in which immature cells which would ordinarily remain in the marrow for a few days longer were liberated early, for studies of the bone marrow have shown that the red blood cells in it grow smaller as they mature.

In the four cases (4b, 5b, 7 and 8) from which red blood cells obtained soon after hemorrhage were measured, it is reasonable to suppose that most of the adult red blood cells had been present before the hemorrhage occurred so that there should be no marked abnormality in their measurements. In these bloods, however, the reticulocytes are relatively small which shows that the mean reticulocyte diameters tend to be low, while the mean adult red blood cell diameters remain at normal values.

In cases 6 and 9 one is justified in assuming that there had been time for some adjustment between the bone marrow and the circulating blood, or, in other words, that practically all of the adult red blood

cells present in the blood had been formed after the hemorrhages so that the parallelism between reticulocytes and adult red blood cells should be partially restored. That there was a trend in this direction is shown by the fact that the ratio between the mean diameters of reticulocytes and adult red blood cells is slightly higher than in the cases where there was not time for adjustment to take place. Although, under prolonged stimulus, the bone marrow was liberating smaller reticulocytes, the adult red blood cells were, by this time smaller also.

From these and other data which have been published on the diameter of red blood cells in chronic "secondary" anemia it is apparent that the reaction of the bone marrow is to form red blood cells which are smaller than normal and which vary more in size as shown by the maximal and minimal measurements that yield the increased 'spread'. Jolly states that there is first a slight increase in mean diameter followed by the decrease observed in this study (12).

A comparison of the measurements of three types of bloods, normal, anemia from loss of blood in which there has been no time for readjustment, and anemia from blood loss in which some readjustment must have taken place, shows that there is a somewhat delayed parallelism between the diameters of reticulocytes and adult red blood cells. It would necessarily follow that, in a patient who has had a single large hemorrhage, there will be a period in which the diameter ratio between reticulocytes and adult red blood cells would be less than that in normal bloods. Once the factor which stimulates the bone marrow to increased production is removed it should begin to form normal blood, including reticulocytes of normal size.

Other observers have reported irregularities in summation-frequency curves of the diameters of red blood cells from cases of pernicious anemia (5) (2). Measurements of 1000 red blood cells from each of two cases of pernicious anemia reported above resulted in curves which conformed to the "Law of Probability" as well as did curves for the diameter of red blood cells from normal bloods. Although the skewness of the curves obtained from measuring 250 red blood cells from the same cases was not marked, the result of measuring the larger number of red blood cells indicates that not all summation frequency curves of the diameters of red blood cells from pernicious anemia bloods are skew curves (see fig. 2).

There is no obvious explanation for the apparently constant increase in the relative diameter of reticulocytes in cases of pernicious anemia having low red blood cell counts. This increase is demonstrable in the diameter measurements, in the values for the ratio between mean reticulocyte and adult red blood cell diameters and is illustrated well by both frequency and summation-frequency curves plotted from the data. It is interesting to note that in the two cases of anemia due to congenital hemolytic jaundice reported above the mean reticulocyte diameter was relatively decreased. There is an excess of blood pigments in the plasma in this disorder as also occurs in pernicious anemia in relapse where the mean reticulocyte diameter is relatively increased. In the congenital disorder the red blood cell diameter measurements were similar to those found in "secondary" anemia, with no increase pigments in the plasma, hence, it is not likely that the presence of pigment has any relation to the diameter of the red blood cells.

#### CONCLUSIONS

- 1 There is a very intimate relationship between the sizes of reticulocytes (immature) and adult red blood cells in the same blood, as indicated by the fact that the shapes of the cell diameter distribution curves are almost identical.

- 2 Reticulocytes of normal bloods average enough larger than adult red blood cells of the same bloods so that the ratio of mean reticulocyte diameter to mean adult red blood cell diameter varies from about 1.12 to 1.15.

- 3 In chronic "secondary" anemias the reticulocytes are relatively smaller than those from normal bloods, so that this ratio varies from 1.06 to 1.10.

- 4 In cases of pernicious anemia having low red blood cell counts (600,000 to 2,200,000 per cubic millimeter) the reticulocytes are much larger than the adult red blood cells, so that the ratio between the mean diameters is between 1.21 and 1.29. This may be characteristic of the blood picture of this disease.

The author wishes to express his appreciation to Dr. George R. Minot for his direction of this work, and to Dr. J. H. Means and F. K. Thomas for their helpful criticism.

## BIBLIOGRAPHY

- 1 Price-Jones, C , *Brit Med J* , 1910, ii, 1418 The Variation in the Sizes of Red Blood Cells.
- 2 Bell, J R , Thomas, F K , and Means, J H , *J Clin Invest* , 1926, iii, 229 Studies on Red Cell Diameter I. In Health and in Pernicious Anemia
- 3 Medearis, D N , and Minot, G R., *J Clin Invest.*, 1927, iii, 541 Studies on Red Blood Cell Diameter II. In Pernicious Anemia, before and during Marked Remission, and in Myelogenous Leukemia.
- 4 Grosh, L C and Stifel, J L , *Arch Int Med* , 1925, xxxvi, 874 The Diameter of the Red Blood Cells in the Differentiation of Anemias.
- 5 Price Jones C , *J Path Bact.*, 1922, xxv, 487 The Diameters of Red Cells in Pernicious Anemia and in Anemia Following Hemorrhage
- 6 Hawes, J B , *Boston Med and Surg J* , 1909, clii, 493 A Study of the Reticulated Red Blood Corpuscle by Means of Vital Staining Methods Its Relation to Polychromatophilia and Stippling
- 7 Key, J A , *Arch Int Med* , 1921, xxviii, 511 Studies on Erythrocytes with Special Reference to Reticulum, Polychromatophilia and Mitochondria
- 8 Gram, H C , *Acta Med Scandinav* , 1927, lxi, 295 On the Size and Form of the Red Cells in Normal and Anemic Cases
- 9 Piiper, A , *J Med Assoc South Africa*, 1928, ii, 483 Liver Diet and the Red Cell Diameter in Addisonian (Pernicious) Anemia
- 10 Minot, G R., and Lee, R I , *Boston Med and Surg J* , 1917, clxxvii, 761 Treatment of Pernicious Anemia especially by Transfusion and Splenectomy
- 11 Whitcher, B R , *Am J Med Sci* , 1925, clxx, 678 Microcytosis in Hemolytic Icterus
- 12 Jolly, J , *Traité Technique d'Hématologie* Vol II, pp 884-885 A Maloine et fils Paris, 1923



## STUDIES ON RED BLOOD CELL DIAMETER

### IV THE DECREASE IN THE MEAN DIAMETER OF THE RETICULOCYTES AND ADULT RED BLOOD CELLS IN PERNICIOUS ANEMIA FOLLOWING LIVER THERAPY

BY GREENE FITZHUGH AND ELBERT LAPSLEY PERSONS

*(From the Medical Service of the Collis P. Huntington Memorial Hospital of Harvard University and the Thorndike Memorial Laboratory of the Boston City Hospital)*

(Received for publication April 15 1929)

Minot and Murphy have shown (1) (2) (3) that in pernicious anemia in relapse one of the first signs of the regular response to adequate liver therapy is a temporary, marked increase of the reticulocytes that develops rapidly. After optimal amounts of liver are given the peak of the reticulocyte increase usually occurs between the sixth and the ninth day, seldom as soon as the fifth or after the tenth day. From the time they have definitely increased until they have returned to a similar level it is rarely less than seven or more than thirteen days, and usually eight to ten days (3).

Persons' studies have shown that in pernicious anemia with a low red blood cell count there is an increase in the mean reticulocyte (immature red blood cell) diameter, as well as in the mean adult red blood cell diameter (4). In view of these findings several sets of measurements have been made of the immature and adult red blood cells from each of nine cases of pernicious anemia over periods ranging from the first twenty-one days to the first six months of remissions induced by the daily ingestion of large amounts of liver. In two of these cases frequent measurements were made during the rise and fall of the reticulocytes.

The measurements on only 5 of the 9 cases are presented in table 1 to conserve space. The data, although obtained at irregular intervals on the other cases, are similar to those presented in graphic form in figure 1. The same method was employed to measure reticulocytes and adult red blood cells as that outlined in a previous paper (5).

TABLE I  
*The blood of 5 cases of pernicious anemia treated with liver*

| Case number | Days | Red blood cells    | Hemo-globin | Reticu-locytes | Measurements of diameters in microns |             |                  |                |               |                | Ratio †<br>reticulocytes<br>adult |
|-------------|------|--------------------|-------------|----------------|--------------------------------------|-------------|------------------|----------------|---------------|----------------|-----------------------------------|
|             |      |                    |             |                | Adult red blood cells                |             |                  |                | Reticulocytes |                |                                   |
|             |      |                    |             |                | Spread                               | Disper-sion | Median diam-eter | Mean diam-eter | Spread        | Mean diam-eter |                                   |
|             |      | millions per cu mm | per cent    | per cent       |                                      |             |                  |                |               |                |                                   |
| 1           | 0*   | 1 4                | 20          | 0 8            | 8 7                                  | 3 4         | 8 7              | 8 2            | 7 5           | 10 6           | 1 29                              |
|             | 10   | 0 9                | 22          | 0 6            | 8 7                                  | 2 7         | 7 9              | 7 7            | 7 5           | 9 9            | 1 28                              |
|             | 16   | 1 7                | 29          | 15 5           | 9 3                                  | 3 0         | 8 2              | 7 9            | 5 6           | 9 1            | 1 14                              |
|             | 23   | 2 7                | 57          | 2 3            | 7 5                                  | 2 2         | 9 0              | 8 7            | 3 1           | 8 8            | 1 02                              |
|             | 32   | 3 6                | 72          | 0 2            | 8 1                                  | 1 9         | 8 7              | 8 3            | 4 3           | 8 7            | 1 04                              |
|             | 57   | 3 8                | 98          | 0 6            | 5 0                                  | 1 4         | 7 8              | 7 5            | 3 7           | 7 8            | 1 04                              |
|             | 126  | 5 0                | 106         | 0 3            | 5 0                                  | 1 5         | 8 2              | 7 9            | 3 7           | 9 4            | 1 18                              |
| 2           | 0*   | 1 2                | 47          | 0 3            | 8 7                                  | 2 4         | 8 2              | 8 0            | 8 1           | 10 0           | 1 25                              |
|             | 7    | 1 4                | 49          | 2 0            | 8 1                                  | 3 0         | 8 1              | 7 7            | 6 2           | 10 0           | 1 30                              |
|             | 24   | 2 5                | 68          | 0 5            | 7 5                                  | 2 5         | 8 7              | 8 4            | 4 3           | 9 7            | 1 16                              |
|             | 56   | 3 5                | 94          | 0 3            | 5 6                                  | 1 7         | 8 0              | 7 7            | 4 3           | 8 8            | 1 14                              |
|             | 179  | 5 3                | 116         | 0 4            | 3 7                                  | 1 2         | 7 4              | 7 0            | 3 1           | 7 97           | 1 13                              |
| 3           | 0    | 1 8                | 48          | 1 4            | 8 1                                  | 1 8         | 8 7              | 8 4            | 6 8           | 10 3           | 1 23                              |
|             | 12   | 1 6                | 35          | 13 1           | 6 8                                  | 2 7         | 7 8              | 7 0            | 5 0           | 8 9            | 1 26                              |
|             | 30   | 2 9                | 66          | 0 6            | 8 1                                  | 2 1         | 8 7              | 8 2            | 3 7           | 9 0            | 1 09                              |
|             | 95   | 4 7                | 82          | 0 2            | 5 0                                  | 1 4         | 8 3              | 8 0            | 3 7           | 8 2            | 1 02                              |
|             | 177  | 6 4                | 94          | 1 0            | 3 7                                  | 1 1         | 7 9              | 7 6            | 3 1           | 8 3            | 1 09                              |
| 4           | 0*   | 2 1                | 47          | 0 9            | 7 5                                  | 2 2         | 8 1              | 7 8            | 5 6           | 9 8            | 1 25                              |
|             | 14   | 2 0                | 65          | 4 0            | 8 1                                  | 2 6         | 9 0              | 8 6            | 6 8           | 10 2           | 1 18                              |
|             | 18   | 3 0                | 62          | 6 4            | 8 1                                  | 2 0         | 8 5              | 8 3            | 4 3           | 8 7            | 1 05                              |
|             | 24   | 3 5                | 75          | 0 8            | 6 8                                  | 2 2         | 8 8              | 8 6            | 4 3           | 8 8            | 1 03                              |
|             | 50   | 5 1                | 110         | 0 05           | 6 8                                  | 1 6         | 7 2              | 6 7            | 3 7           | 7 6            | 1 13                              |
|             | 77   | 5 5                | 109         | 0 3            | 5 6                                  | 1 2         | 8 1              | 7 9            | 3 7           | 8 5            | 1 08                              |
|             | 166  | 5 7                | 110         | 0 2            | 4 3                                  | 1 2         | 7 8              | 7 6            | 3 7           | 8 5            | 1 12                              |
| 5           | 0*   | 0 7                |             | 3 3            | 10 8                                 | 2 5         | 8 6              | 8 6            | 8 4           | 10 3           | 1 19                              |
|             | 3    | 0 8                |             | 2 9            | 9 6                                  | 3 1         | 8 7              | 8 5            | 8 4           | 10 8           | 1 26                              |
|             | 5    | 0 9                |             | 6 2            | 9 6                                  | 2 7         | 8 7              | 8 5            | 9 0           | 10 6           | 1 25                              |
|             | 6    | 0 9                |             | 12 0           | 9 6                                  | 3 3         | 8 3              | 8 1            | 9 0           | 10 6           | 1 32                              |
|             | 8    |                    |             | 50 9           | 7 2                                  | 3 4         | 8 4              | 8 2            | 8 4           | 9 3            | 1 14                              |
|             | 10   | 1 4                |             | 36 4           | 9 0                                  | 2 4         | 8 7              | 8 6            | 6 0           | 9 2            | 1 07                              |
|             | 12   |                    |             | 16 3           | 8 4                                  | 2 0         | 8 7              | 8 7            | 6 0           | 9 2            | 1 05                              |
|             | 19   | 2 4                |             | 4 1            | 7 8                                  | 1 8         | 8 5              | 8 6            | 4 2           | 8 7            | 1 01                              |
|             | 21   | 2 8                |             | 0 9            | 8 4                                  | 1 9         | 8 4              | 8 3            | 4 2           | 8 8            | 1 06                              |

\* Case 1 Liver started on 6th day    Case 2 Liver started on 4th day    Case 4 Liver started on 8th day    Case 5 Liver started on 2nd day

† The second decimal place is omitted in the table, but was used to determine the ratio  $\frac{\text{reticulocytes}}{\text{adult}}$

Cases 1 to 4 inclusive were among the earliest cases treated by Minot and Murphy. They received only about 150 grams of cooked liver a day and hence their response was definitely slower than occurs in most cases given larger amounts of liver, kidney, or full doses of potent liver extract (3).

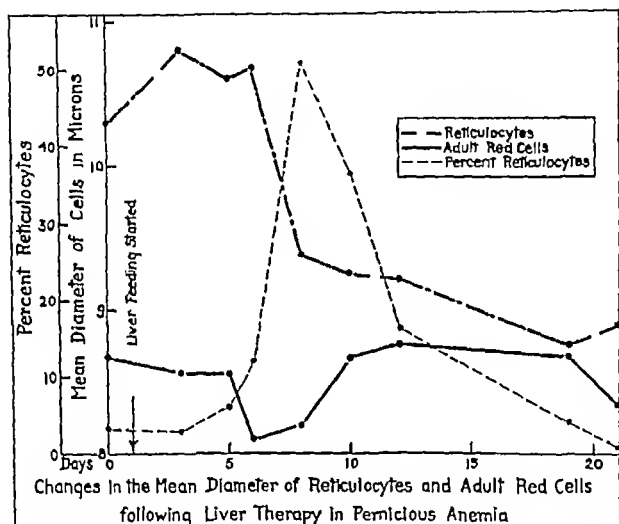


FIG 1 THE DECREASE OF THE MEAN DIAMETER OF THE RETICULOCYTES AND ADULT RED BLOOD CELLS IN RELATION TO THE TEMPORARY INCREASE OF RETICULOCYTES PRODUCED BY LIVER FEEDING IN PERNICIOUS ANEMIA

The curves in figure 1 which were constructed from the data presented for case 5 (table 1) are comparable to curves plotted for the other cases. The patient received daily 250 grams of raw liver pulp. During the first five days of liver feeding there was a slight rise in the mean diameter of the reticulocytes, but no significant change in the diameter of the adult red blood cells. On the fifth day the rise in the reticulocytes began and reached a peak on the eighth day, gradually

falling to near normal by the nineteenth day. From the fifth to the nineteenth day the most important changes in red blood cell size occurred. They were (1) A marked drop in mean reticulocyte diameter from the sixth to the eighth day as the reticulocytes rose. (2) A gradual decrease in mean reticulocyte diameter from the eighth day onwards. (3) A distinct rapid drop in mean adult red blood cell diameter from the fifth to the sixth day followed by an increase from the eighth to the twelfth day to within 0.10 micron of the original size. (4) A very gradual decrease in mean adult red blood cell diameter from the twelfth day onwards.

In cases followed for longer periods of time the mean reticulocyte and adult red blood cell diameters decreased gradually so that normal proportions were usually found when the red blood cell count reached normal figures. Not only did the actual diameter measurements become normal but the abnormally high ratio between the diameters of the reticulocytes and adult red blood cells approached normal, as the data in the last column of table 1 shows. Both the spread (difference in diameter between the smallest and largest red blood cell found) and the dispersion (difference between the 16 per cent and 84 per cent grade taken from the curve of red blood cell size plotted on arithmetic probability paper) also approached normal as shown in table 1. Davidson and McCrie (7), using a different method to measure red blood cells, question the findings of Medearis and Minot who state that "The mean diameter of the red blood cells in pernicious anemia may become normal in patients improved by a special diet rich in liver when the red blood cell count increases to between 4,200,000 and 6,300,000 per cubic millimeter." Our findings are in accord with the statement of Medearis and Minot (6). It would appear that one of the signs to indicate that the patient has received and is receiving an adequate amount of anti-pernicious anemia substance is that the red blood cell diameters are normal or essentially normal.

It is not likely that the initial rise in the mean diameter of the reticulocytes which occurred before the rise in the number of these cells is significant. The pronounced drop in mean reticulocyte diameter and the less marked drop in mean adult red blood cell diameter, which occurred as the reticulocytes rose to a peak, are

outstanding rapid changes. The subsequent rise in mean adult red blood cell diameter coincident with the fall in the number of reticulocytes was detected in several cases. It is apparently a change of short duration and lasted only four days in case 5. It was an unexpected finding the cause of which is not evident. The subsequent slow decrease in mean reticulocyte and adult red blood cell diameter, which continued until normal proportions were found, when the red blood cell count approached normal, adds further evidence to that in the literature that continued adequate liver therapy can permit the bone marrow in pernicious anemia to manufacture red blood cells of normal size.

#### CONCLUSIONS

Measurements of the diameters were made of immature (reticulocytes) and adult red blood cells from 9 cases of pernicious anemia during treatment with liver.

As the reticulocytes increased there occurred an initial drop in the mean adult red blood cell diameter which soon rose again almost to its original level. The size remained approximately the same while the red blood cells increased about 1 million per cubic millimeter and then progressively decreased to about normal as the red blood cell count rose above 4.5 million per cubic millimeter.

The mean reticulocyte diameter decreased markedly as the reticulocytes rapidly rose. It then continued to decrease slowly becoming normal or essentially so when the red blood cells approached 5 million per cubic millimeter.

Decrease to normal values in the mean reticulocyte and adult red blood cell diameters in pernicious anemia can result from continued adequate liver therapy causing changes in the bone marrow.

The authors wish to express their appreciation to Dr. George R. Minot for his direction of this work, and to Dr. J. H. Means and F. K. Thomas for their helpful criticism.

#### BIBLIOGRAPHY

1. Minot, G. R., and Murphy, W. P., *J. Am. Med. Assoc.*, 1926, *lxxxvii*, 470.  
Treatment of Pernicious Anemia by a Special Diet.

- 2 Minot, G R , and Murphy, W P , J Am Med Assoc , 1927, lxxxix, 759  
A Diet Rich in Liver in the Treatment of Pernicious Anemia
- 3 Minot, G R , Murphy, W P , and Stetson, R P , Am J Med Sci , 1928,  
clxxv, 581 The Response of the Reticulocytes to Liver Therapy Particu-  
larly in Pernicious Anemia
- 4 Persons, E L , J Clin Invest , 1929, vii, 615 Studies on Red Cell Diameter  
III The Relative Diameter of Immature (Reticulocytes) and Adult Red  
Blood Cells in Health and Anemia, Especially in Pernicious Anemia
- 5 Bell, J R , Thomas, F K , and Means, J H , J Clin Invest , 1926, iii, 229  
Studies on Red Cell Diameter I In Health and in Pernicious Anemia
- 6 Medearis, D N , and Minot, G R , J Clin Invest , 1927, iii, 541 Studies on  
Red Blood Cell Diameter II In Pernicious Anemia, Before and During  
Marked Remission, and in Myelogenous Leukemia
- 7 Davidson, L S P , and McCrie, J G , Lancet, 1928, ii, 1014 The Phenom-  
enon of Reticulation and the Alteration in Size of the Red Blood Cor-  
puscles after Liver Therapy

## INDEX TO VOLUME VII

- Acid Base Balance, Effect of Sodium Chloride, Ammonium Chloride and Sodium Bicarbonate on, in a Case of Chronic Nephritis Fuller Albright and Walter Bauer, 465
- Albright, Fuller and Bauer, Walter, The Action of Sodium Chloride, Ammonium Chloride, and Sodium Bicarbonate on the Total Acid Base Balance of a Case of Chronic Nephritis with Edema, 465
- Albright, Fuller, Bauer, Walter, Ropes, Marion and Aub, Joseph C., Studies of Calcium and Phosphorus Metabolism. IV The Effects of the Parathyroid Hormone, 139
- Albright, Fuller and Ellsworth, Read, Studies on the Physiology of the Parathyroid Glands. I. Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism 183
- Albright, Fuller See Bauer Walter
- Alexander, H. L., 512
- Allan, Frank N., 309
- Allen, Edgar V., 309
- Alloway, James L., 522
- American Society for Clinical Investigation, Proceedings of Twenty first Annual Meeting Held in Atlantic City, N. J., May 6 1929, 487
- Andrus, E. Cowles, 491
- Anemia, Decrease in Red Blood Cell Diameter in Pernicious Anemia Following Liver Therapy Greene Fitz Hugh and Elbert Lapsley Persons, 631
- Anemia, Red Blood Cell Diameter in, Elbert Lapsley Persons, 615
- Anemia, Splenic Blood Volume Preceding and Following Splenectomy in, Herbert Z. Giffin, George E. Brown and Grace M. Roth, 283
- Aub Joseph C, Bauer, Walter, Heath Clark and Ropes, Marion, Studies of Calcium and Phosphorus Metabolism. III. The Effects of the Thyroid Hormone and Thyroid Disease 97
- Aub, Joseph C. See Albright, Fuller and Bauer Walter
- Austin, J. H., 504
- Austin J. Harold and Sunderman, F. William, Studies of Serum Electrolytes V Urinary Electrolyte Excretion in Pneumonia, 333
- Baker Benjamin M., 526
- Baker John C., 524
- Barker, Paul S., 518
- Barr, David P., 306 310 and 498
- Base Metabolism in Pneumonia. T. S. Wilder and T. G. H. Drake, 353
- Bauer, Walter, Albright, Fuller and Aub Joseph C., Studies of Calcium and Phosphorus Metabolism. II. The Calcium Excretion of Normal Individuals 75
- Bauer Walter See Albright, Fuller and Aub Joseph C
- Bishop, F. W., 521
- Blake, Francis G., 519
- Blankenhorn, M. A., 310
- Blood Cell Diameter, Decrease of, in Reticulocytes and Adult Red Blood Cells in Pernicious Anemia Following Liver Therapy Greene Fitz Hugh and Elbert Lapsley Persons, 631
- Blood Cell Diameter of Immature and Adult Red Blood Cells in Health and Anemia, Especially in Pernicious Anemia. Elbert Lapsley Persons, 615
- Blood Cells, Red, Sedimentation Rate of, M. Dorothy Rourke and E. D. Plass, 365

- Blood Flow Through Lungs, Velocity of, Effect of Digitalis Bodies on, Soma Weiss and Herrmann L Blumgart, 11
- Blood Serum, Surface Tension of, Henry N Harkins and William D Harkins, 263
- Blood Volume in Infants, Determination of, by the Carbon Monoxide Method Rustin McIntosh, 203
- Blood Volume, Preceding and Following Splenectomy in Hemolytic Icterus and Anemia Herbert Z Giffin, George E Brown and Grace M Roth, 283
- Bloomfield, Arthur L See Polland, W Scott
- Blumgart, Herrmann L, 493
- Blumgart, Herrmann L See Weiss, Soma
- Boas, Ernst P, 491
- Bohning, Ann, 518
- Borsook, Henry, 510
- Braley, Allen G See Thompson, Willard Owen
- Briggs, A P, 506
- Bromer, Albert W, 487
- Broun, G O, 506
- Brown, George E, 311
- Brown, George E See Giffin, Herbert Z
- Brown, Madelaine R, 520
- Brunsting, Louis A and Sheard, Charles, The Color of the Skin as Analyzed by Spectrophotometric Methods II The Role of Pigmentation, 575  
The Color of the Skin as Analyzed by Spectrophotometric Methods III The Role of Superficial Blood, 593
- Brunsting, Louis A See Sheard, Charles
- Bulger, Harold A., 306 and 498
- Bumstead, John H., 519
- Burwell, C Sidney and Smith, W Carter, The Output of the Heart in Patients with Abnormal Blood Pressures, 1
- Calcium and Phosphorus Studies on a Case of Idiopathic Hyperparathyroidism. Fuller Albright and Read Ellsworth, 183
- Calcium Excretion of, in Normal Individuals and in a Case of Pregnancy Walter Bauer, Fuller Albright and Joseph C Aub, 75
- Calcium Metabolism, Effects of Thyroid on, Joseph C Aub, Walter Bauer, Clark Heath and Marion Ropes, 97
- Calcium Metabolism of, Effects of Parathyroid Hormone on, Fuller Albright, Walter Bauer, Marion Ropes and Joseph C Aub, 139
- Capillaries During Passive Congestion, Influence of the Sympathetic Nervous System on, J Hamilton Crawford, 527
- Capps, Joseph A, 505
- Cardiac Output in Patients with Abnormal Blood Pressures, C Sidney Burwell and W Carter Smith, 1
- Cardiac Size, Effects of Exercise on, Harold J Stewart, 339
- Cardiac Systole, Duration of, as Indicated by Length of Q-T Interval of the Electrocardiogram Paul D White and Seeley G Mudd, 387
- Carter, Edward P, 491 and 526
- Central Society for Clinical Research, Proceedings of First Meeting Held in Chicago, Ill, November 23, 1928, 303
- Chesney, Alan M, 517
- Chloride Balance in Pneumonia F William Sunderman, 313
- Chloride Metabolism in Pneumonia T S Wilder and T G H Drake, 353
- Cobb, Stanley, 517
- Cohen, Archibald C See Thompson, Willard Owen
- Collins, Leon H, Jr, 513
- Conner, H M, 310
- Crawford, J Hamilton, The Influence of the Sympathetic Nervous System on the Capillaries During Passive Congestion, 527
- Curtis, A C, 518
- Curtis, A C, Wile, U J and Eckstein, H C, The Involution of Cutaneous Xanthomata Caused by Diets Low in Calories, 249
- Cutler, Elliott C, 501
- Denck, Clifford L, 514

- Digitalis Bodies, Effect of on Velocity of  
Blood Flow Through the Lungs Soma  
Weiss and Herrmann L Blumgart 11
- Dixon, Henry H 306
- Dochez, A. R., 502
- Drake, T G H See Wilder, T S
- Drinker, Philip and Shaw, Louis A, An  
Apparatus for the Prolonged Adminis-  
tration of Artificial Respiration. I  
A Design for Adults and Children, 229
- DuBois, E. F, 499
- Duden, Charles, 310
- Eckstein, H. C. See Curtis, A C
- Electrocardiogram, Q-T Interval of, Paul  
D White and Seeley G Mudd, 387
- Electrolyte Excretion in Pneumonia J  
Harold Austin and F William Sunder-  
man, 333
- Ellis, Laurence B, 490
- Ellsworth, Read See Albright, Fuller
- Ernstene, A. Carlton, 493 and 511
- Evans, Frank A 516
- Fairhall, Lawrence T and Hoyt, Lyman  
H., The Excretion of Zinc in Health  
and Disease, 537
- Falk, E., 499
- Farquharson, Ray 510
- Field, Henry, Jr, 497
- Fitz Hugh, Greene and Persons, Elbert  
Lapsley, Studies on Red Blood Cell  
Diameter IV The Decrease in the  
Mean Diameter of the Reticulocytes  
and Adult Red Blood Cells in Perni-  
cious Anemia Following Liver Therapy,  
631
- Forbes, H S, 517
- Fremont Smith, Frank, 489
- Fulton Marshall N, 514
- Garcia, O 310
- Giffin, H Z. 310
- Giffin, Herbert Z. Brown, George E and  
Roth Grace M Blood Volume Pre-  
ceding and Following Splenectomy  
in Hemolytic Icterus and Splenic  
Anemia, 283
- Glover, E C., 509
- Goldblatt, Harry, 505
- Gordon Burgess, 524
- Gorham, L W 504
- Goulding, A M, 510
- Grabfield, G P, 495
- Graham, Duncan, 510
- Grove, Edward, 305
- Haden Russell L 521
- Hamburger, Walter W, 519
- Hansen Pruss, Oscar C, Longcope, War-  
field T and O'Brien, D P, Skin  
Reactions to Filtrates of Hemolytic  
Streptococci in Acute and Subacute  
Nephritis, 543
- Harkins, Henry N and Harkins, William  
D, The Surface Tension of Blood  
Serum, and the Determination of the  
Surface Tension of Biological Liquids,  
263
- Harkins, William D See Harkins, Henry  
N
- Harrison T R, 494
- Hayman, J M, Jr, 488
- Henth Clark. See Aub, Joseph C.
- Hemoglobinuria, Paroxysmal Observations,  
on G M Mackenzie 27
- Herrmann Louis G, 501
- Histamine Stimulation Effect of, on  
Pepsin in Gastric Juice W Scott  
Pollard and Arthur L Bloomfield, 57
- Holmes, William H., 305
- Horten Bayard T 311
- Howe, Marion, 495
- Hoyt, Lyman H. See Fairhall, Lawrence  
T
- Hunt H D, 504
- Isaacs Raphael, 312 and 520
- Jackson, Henry, Jr, 509
- Jacobson, B M, 511
- Jaundice, Hemolytic, Blood Volume Pre-  
ceding and Following Splenectomy in,  
Herbert Z. Giffin, George E Brown  
and Grace M Roth, 283
- John, Henry J, 309
- Katz, Louis N, 304
- Keith, N M, 495
- Kneeland, Yale, Jr, 502
- Kountz, W B, 512
- Leiter, Louis 493
- Lemon, Willis S, 499

- Lennox, William G , 517  
 Levine, S A , 511  
 Loebel, R O , 507  
 Longcope, Warfield T See Hansen-Pruss, Oscar C  
 Luten, Drew, 305  
 McClellan, W S , 499  
 McCluggage, H B , 516  
 McGuire, Johnson, 304  
 McIntosh, Rustin, The Determination of the Circulatory Blood Volume in Infants by the Carbon Monoxide Method, 203  
 McLean, Franklin C , 493  
 McPhedran, F Maurice, 512  
 Mackenzie, G M , Observations on Paroxysmal Hemoglobinuria, 27  
 Makepeace, Alexander W , 489  
 Mettler, Stacy R , 510  
 Meyer, O O , 303 and 520  
 Middleton, William S , 303 and 520  
 Miller, A J , 307  
 Miller, George H , 506  
 Minot, George R , 510  
 Moore, Joseph Earle, 521  
 Morgan, Hugh J , 522  
 Morrison, L Raymond, 489  
 Mudd, Seeley G See White, Paul  
 Myers, J A , 307  
 Myxedema, Calorigenic Action of Thyroxin in, at Different Levels of Basal Metabolism Willard Owen Thompson, Phebe K. Thompson, Allen G Brailey and Archibald C Cohen, 437  
 Nephritis, Acute and Subacute, Skin Reactions of Hemolytic Streptococci in, Oscar C Hansen-Pruss, Warfield T Longcope and D P O'Brien, 543  
 Nephritis, Chronic, with Edema, Action of Sodium Chloride, Ammonium Chloride and Sodium Bicarbonate on the Total Acid-Base Balance of, Fuller Albright and Walter Bauer, 465  
 Nervous System, Sympathetic, Influence of, on the Capillaries During Passive Congestion, J Hamilton Crawford, 527  
 Newburgh, L H , 518  
 Nitrogen Balance in Pneumonia, F William Sunderman, 313  
 O'Brien, D P See Hansen-Pruss, Oscar C  
 O'Donovan, C , Jr , 522  
 Ottenberg, Reuben, 515  
 Palmer, Walter Lincoln, 505  
 Parathyroid Hormone, Effects of Calcium and Phosphorus Metabolism Fuller Albright, Walter Bauer, Marion Ropes and Joseph C Aub, 139  
 Parathyroid Physiology Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism Fuller Albright and Read Ellsworth, 183  
 Parker, Frederic, Jr , 509  
 Pepsin, Method for Estimation of, Arthur L Bloomfield and W Scott Pollard, 45  
 Pepsin, Quantitative Measurements of, in Gastric Juice Before and After Histamine Stimulation W Scott Pollard and Arthur L Bloomfield, 57  
 Perry, Margaret C , 310  
 Persons, Elbert Lapsley, Studies on Red Blood Cell Diameter III The Relative Diameter of Immature (Reticulocytes) and Adult Red Blood Cells in Health and Anemia, Especially in Pernicious Anemia, 615  
 Persons, Elbert Lapsley See Fitz Hugh, Greene  
 Phosphorus and Calcium Studies on a Case of Idiopathic Hypoparathyroidism Fuller Albright and Read Ellsworth, 183  
 Phosphorus Metabolism, Effects of Parathyroid Hormone on, Fuller Albright, Walter Bauer, Marion Ropes and Joseph C Aub, 139  
 Phosphorus Metabolism, Effects of Thyroid on, Joseph C Aub, Walter Bauer, Clark Heath and Marion Ropes, 97  
 Pigmentation, Effect of, on Skin Color as Analyzed by Spectrophotometric Methods Louis A Brunsting and Charles Sheard, 575  
 Pilcher, Cobb, 494  
 Pilot, I , 307

- Plass, E D See Rourke, M Dorothy  
Pneumonia Chloride and Nitrogen  
Balances and Weight Changes in, F  
William Sunderman, 313  
Pneumonia, Metabolism of Chloride and  
Total Fixed Base In and their Rela-  
tion to Salt and Water Retention T  
S Wilder and T G H Drake 353  
Pneumonia, Urinary Electrolyte Excretion  
in J Harold Austin and F William  
Sunderman 333  
Pollard W Scott and Bloomfield, Arthur  
L. A Quantitative Method for the  
Estimation of Pepsin, 45  
Pollard, W Scott and Bloomfield Arthur  
L, Quantitative Measurements of  
Pepsin in Gastric Juice Before and  
After Histamine Stimulation, 57  
Poole, Allan K., 519  
Pregnancy, Calcium Excretion in Walter  
Bauer, Fuller Albright and Joseph  
C Aub 75  
Proceedings of the First Meeting of the  
Central Society for Clinical Research  
Held in Chicago, Ill. November 23,  
1928, 303  
Proceedings of the Twenty first Annual  
Meeting of the American Society for  
Clinical Investigation Held in Atlantic  
City, N J, May 6, 1929, 487  
Rentschler, E. B, 311 and 516  
Respiration Artificial An Apparatus for,  
Phillip Drinker and Louis A Shaw,  
229  
Reznikoff, Paul, 515  
Richardson H B, 507  
Riddle, Matthew C., 498  
Riecker Herman H., 497  
Ritchie, E. K., 521  
Robinson Harry M, 521  
Ropes, Marion. See Albright, Fuller and  
Aub Joseph C.  
Roth, Grace M See Giffin, Herbert Z  
Rourke M Dorothy and Plass, E. D, An  
Investigation of Various Factors which  
Affect the Sedimentation Rate of the  
Red Blood Cells, 365  
Rowntree, L G, 311 and 516  
Sedimentation Rate of the Blood Cells.  
M Dorothy Rourke and E D Plass,  
365  
Shaw, Louis A. See Drinker, Philip  
Sheard, Charles and Brunsting, Louis A,  
The Color of the Skin as Analyzed by  
Spectrophotometric Methods I. Ap-  
paratus and Procedure 559  
Sheard, Charles See Brunsting, Louis A.  
Shohl, A. T, 505  
Shorr, E 507  
Siegel, Mortimer L., 304  
Singer Harry A., 308  
Skin, Color of, as Analyzed by Spectro-  
photometric Methods. Charles  
Sheard and Louis A Brunsting, 559  
Skin Color of, As Analyzed by Spectro-  
photometric Methods as Influenced  
by Superficial Blood Louis A Brun-  
sting and Charles Sheard, 593  
Skin, Color of, Role of Pigmentation  
Louis A. Brunsting and Charles  
Sheard 575  
Smith David T, 504  
Smith Fred M, 506  
Smith, W Carter See Burwell, C. Sidney  
Spencer H J 499  
Starr, Isaac, Jr, 513  
Starr, Paul, 306  
Stewart, Harold J, The Effect of Exercise  
on the Size of Normal Hearts and of  
Enlarged Hearts of Dogs, 339  
Strang, J M 516  
Streptococci, Hemolytic, Skin Reactions to  
Filtrates of, in Acute and Subacute  
Nephritis, Oscar C. Hansen Pruss,  
Winfield T Longcope and D P  
O'Brien, 543  
Strouse, Solomon, 524  
Sturgis, Cyrus C 312 and 498  
Sunderman F W, 504  
Sunderman F William, Studies of Serum  
Electrolytes. IV The Chloride and  
Nitrogen Balances and Weight  
Changes In Pneumonia 313  
Sunderman, F William See Austin,  
J Harold  
Surface Tension of Blood Serum and

- Biological Liquids Henry N Harkins and William D Harkins, 263
- Sutton, Dan C, 305
- Thalheimer, William, 310
- Thomas, William A., 312
- Thompson, Phebe K See Thompson, Willard Owen
- Thompson, Willard Owen, Thompson, Phebe K, Brailey, Allen G and Cohen, Archibald C, The Calorigenic Action of Thyroxin at Different Levels of Basal Metabolism in Myxedema, 437
- Thyroid Hormone and Thyroid Disease, Calcium and Phosphorus Metabolism in, Joseph C Aub, Walter Bauer, Clark Heath and Marion Ropes, 97
- Thyroxin, Action of, at Different Levels of Basal Metabolism in Myxedema Willard Owen Thompson, Phebe K Thompson, Allen G Brailey and Archibald C Cohen, 437
- Trask, James D, 522
- Trimble, W H, 513
- Vanzant, Frances R, 311 and 516
- Wakefield, E G, 495
- Wang, C C, 524
- Warren, S L, 521
- Water Retention in Pneumonia T S Wilder and T G H Drake, 353
- Wearn, Joseph T, 487
- Webster, Bruce, 517
- Weiss, Soma, 490
- Weiss, Soma and Blungart, Herrmann L, The Effect of the Digitalis Bodies on the Velocity of Blood Flow Through the Lungs and on Other Aspects of the Circulation A Study of Normal Subjects and Patients with Cardiovascular Disease, 11
- West, Randolph, 495
- White, Paul D and Mudd, Seeley G, Observations on the Effect of Various Factors on the Duration of the Electrical Systole of the Heart as Indicated by the Length of the Q-T Interval of the Electrocardiogram, 387
- Wilder, T S, and Drake, T G H, Metabolism of Chloride and Total Fixed Base on Pneumonia and its Relation to Salt and Water Retention, 353
- Wile, U J See Curtis, A C
- Wilbams, John R., 524
- Willius, Frederick A, 308
- Wilson, Frank N, 518
- Winters, Mary E, 497
- Wolff, H G, 517
- Xanthomata, Cutaneous, Involution of, Caused by Diets Low in Calories A. C Curtis, U J Wile and H C Eckstein, 249
- Youmans, John B, 513
- Zinc, Excretion of, in Health and Disease. Lawrence T Fairhall and Lyman H Hoyt, 537
- Zschiesche, Louise J, 487

